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SEPTEMBER 1954
VOLUME 58 NUMBER 3

Published Monthly by
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

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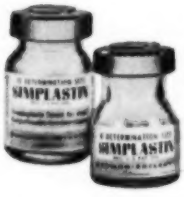

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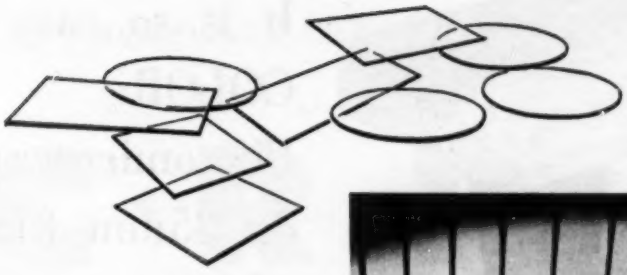

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VOLUME 58 SEPTEMBER 1954 NUMBER 3

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TISSUE DISTRIBUTION OF INJECTED RADIOACTIVE COLLOIDAL CHROMIC PHOSPHATE ($CrP^{32}O_4$)

JAMES B. McCORMICK, M.D.
GEORGE MILLES, M.D.
BERTHA JAFFE, M.D.
AND
LINDON SEED, M.D.
CHICAGO

THE PRESENT experiments were planned to accumulate data on the distribution and localization of colloidal chromic phosphate tagged with radioactive phosphorus ($Cr\ P^{32}O_4$) when injected into animals. Our ultimate purpose is to explore the possibility of using such a colloid suspension in the treatment of neoplasms by injecting the radioactive material directly into the tumor. Radiophosphorus, P^{32}, has a 14.3 day half-life and emits only beta rays with a maximum energy of 1.7 mev (million electron volts). Since P^{32} has no gamma emission, it is a convenient isotope to handle. Most of the beta emissions can be screened out by surrounding a syringe with only a 2 cm. layer of Lucite. The half-life of 14.3 days permits considerable ionizing radiation from a relatively small amount of isotope. In these respects P^{32} would be much superior to radioactive Au^{198}, which has a component of gamma radiation and a half-life of only 2.7 days.

MATERIALS AND METHODS

The chromic phosphate was prepared by Abbott Laboratories under the direction of Dr. Donalee Tabern and furnished in two particle sizes. In one suspension the particles varied from 2 to 4 μ in diameter; in the other the particles had an average diameter of 0.2 μ. The colloidal particles were suspended in a 2% pectin solution. The specific activity was such that 1 mc. was carried on 10 mg. of chemical chromic phosphate.

Four sets of experiments were carried out to ascertain the fate of the radioactive substance injected into the animals: 1. The isotope was injected intravenously into rabbits and rats and the organs were analyzed. 2. Rabbits received subcutaneous injections in the ear. 3. One series of rats received intramuscular injections in the pectoral region. 4. Another series of rats received injections into one testis.

INTRAVENOUS INJECTION OF $Cr\ P^{32}O_4$

Fifty microcuries (50 μc) of radioactive chromic phosphate was given as a single injection into the marginal ear vein of 12 mature male and female rabbits. They were killed at weekly intervals. The wet organs were weighed and dissolved in 2 N NaOH solution for analysis. One-milliliter aliquots of the dissolved organs were placed in stainless steel planchets, dried under an infrared light, and counted under

Chief of Laboratory Service, United States Public Health Hospital (Dr. McCormick).

From the Isotope Laboratory and the Pathology Department, Augustana Hospital; supported by funds furnished by the Nelson M. Percy Research Foundation.

a mica end-window Geiger tube. The tube was calibrated by placing a known quantity of P^{32} in an identical solution of the same organ taken from a normal animal. Quantitative analysis was made of the radioactivity of the liver, spleen, lymph

TABLE 1.—*Rabbit Tissue Distribution One Week After Intravenous Injection of 53 μc of Cr $P^{32}O_4$.**

Organ	Wt., Gm.	μc/Gm.	Total μc	% of Total
Spleen	1.15	1.76	2.04	3.85
Liver	105.3	0.46	48.37	91.26
Lymph nodes	.....	0.079	.....	....
Heart	6.92	0.037	0.256	0.48
Lungs	11.78	0.015	0.185	0.35
Bone	.....	0.008	.....	....
Adrenals	0.83	0.007	0.006	0.01
Kidney	18.49	0.008	0.044	0.08
Salivary gland	1.95	0.0014	0.008	0.005
Blood plasma, 1 cc	.....	0.011	.....	....
Urine, 1 cc	.....	0.008	.....	....
Thyroid, ovaries	.....	.....	.....	0
Brain	.....	.....	.....	0

* Total accounted for, 96.04%. Values are corrected for decay.

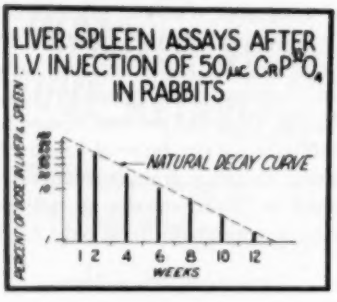

Figure 1

nodes, kidneys, brain, lungs, heart, and bone marrow in the first animal, killed after one week (Table 1). Approximately 95% of the 50 μc of injected radioactive material was recovered from the liver and spleen. Lymph nodes, lungs, kidneys, and other tissues contained negligible amounts. Urine, collected during the week of observation, contained insignificant amounts of radioactivity. Since nearly all of the radioactivity localized in the liver and spleen, only these two organs were analyzed in the remaining animals. The total quantity of radioactivity found in the liver and spleen of each rabbit at intervals of up to 14 weeks is charted against a curve representing the rate of physical decay of P^{32}. These two lines closely approximate each other throughout the term of the experiment, indicating that the chromic phosphate not only localized in the liver and spleen, but was held there throughout the 14 week period (Fig. 1).

Anatomical Findings.—No gross changes were apparent at autopsy, except for the presence of up to 6 cc. of ascitic fluid in animals killed from the 4th to the 14th week. Microscopic examination of the liver and spleen revealed particles in the reticuloendothelial cells which gave a negative Prussian blue reaction and were

assumed to be chromic phosphate. One animal was given 250 mg. of nonradioactive chromic phosphate as a single intravenous injection (500 times the amount of chromic phosphate injected intravenously as carrier for the P^{32}). This animal died after five days. Ninety per cent of the chromic phosphate was accounted for by chemical analysis in the liver and spleen. Microscopically, particles were identified in great abundance in these organs, and focal necrosis of the kidney cortex appeared to be due to vascular occlusion by emboli of chromic phosphate.

In an attempt to determine the maximum radiation tolerance of the liver and spleen, 20 to 250 μc of Cr $P^{32}O_4$ was given intravenously to 17 150-gm. rats. Analyses of radioactivity and histologic studies were made during periods up to 77 days. Radiation dosage up to 25,461 rep caused no gross or histologic changes in the liver or spleen. Assay data are presented in Table 2.

Conclusions and Comment.—Radioactive phosphorus given intravenously in the form of colloid, Cr $P^{32}O_4$, with particle sizes of 2 to 4 μ localized in the liver and spleen and was held in these organs. There was no evidence of breakdown of the chromic phosphate or its excretion from the body.

TABLE 2.—*Distribution of Radioactive Chromic Phosphate Injected Intravenously**

Dose	Organ	25 Days		31 Days		37 Days		60 Days		71 Days		77 Days	
		μc/Gm.	Rep	μc/Gm.	Rep	μc/Gm.	Rep	μc/Gm.	Rep	μc/Gm.	Rep	μc/Gm.	Rep
20 μc	Liver	1.34	1,186	....	.....	....	.....	3.43	3,035	....	.....	....	.....
	Spleen	0.23	203	....	.....	....	.....	0.52	460	....	.....	....	.....
30 μc	Liver	1.57	1,389	....	.....	....	.....	0.56	495	....	.....	7.69	6,805
	Spleen	0.35	309	....	.....	....	.....	0.08	17	....	.....	0.24	212
40 μc	Liver	0.116	106	....	.....	....	.....	4.1	3,628	6.76	5,982	11.3	10,000
	Spleen	0.01	8.8	....	.....	....	.....	0.17	150	0.40	354	0.168	150
80 μc	Liver	4.22	3,734	....	.....	....	.....	7.6	6,726	15.77	13,956	....	.....
	Spleen	0.992	876	....	.....	....	.....	0.21	185	0.225	203	....	.....
150 μc	Liver	....	.....	....	.....	30.5	34,957	15.51	13,726	....	.....	....	.....
	Spleen	....	.....	....	.....	2.42	2,141	....	.....	....	.....	....	.....
250 μc	Liver	....	.....	28.77	25,461	1.27	10,858	26.0	23,010	....	.....	....	.....
	Spleen	....	.....	0.714	628	4.5	3,085	11.04	9,770	....	.....	....	.....

* Difference in assay quantities and initial dosage caused by loss during injections.

Herve and Govaerts,[9] Gabrieli,[4] and Jones and associates [11] also found that radioactive chromic phosphate was fixed to a large extent in the liver and spleen, but that some also went to the bone marrow and lungs.

Numerous investigators * have found that colloidal gold, Au^{198}, when injected intravenously, is accumulated to a large extent by the liver and spleen. After injection, the colloid is cleared from the blood stream, apparently by one circulation through the liver.[17] Its half-life in the blood stream has been calculated by Sheppard, Jordan, and Hahn [17] at from 0.9 to 2 minutes and by Zilversmit, Boyd, and Brucer [25] at from 1.5 to 13 minutes. Larger particles are removed more rapidly than smaller ones.

The particles of colloidal Au^{198} used by most investigators are much smaller than those of chromic phosphate, being in the neighborhood of 0.03 μ diameter and varying from 0.001 to 0.5 μ.

Gabrieli calculated the half-life of colloidal chromic phosphate in the blood stream as from 0.45 to 1.25 minutes. Colloid particles up to 30 μ in diameter pass through

* References 2, 3, 5, 7, 8, 13, and 19.

the lung, but particles larger than 30 μ are removed from the circulation by the lung. The behavior of different colloid particles of the same size is not necessarily identical.[18]

Jones, Wrobel, and Lyons [11] were the first to investigate chromic phosphate. They injected the substance intravenously into mice and found 59 to 81% of the dose in the liver, 4.3 to 14% in the spleen, and 0.6 to 6.6% in the lung. In 2 out of 10 mice receiving 300 μc intravenously, hepatomas developed, a tumor never seen in the strain of mice used. The distribution in a dog, given a single intravenous injection, was as follows: liver, 90.2%; spleen, 6.3%; lungs, 1.6%; bone marrow, lymph nodes, pituitary, and all other organs, 1.9%.

For all practical purposes one can assume that if radioactive colloidal chromic phosphate enters the blood stream directly or indirectly, it is fixed in the liver and spleen and the ionizing radiation is expended in these organs. Hahn, Jackson, and Goldie [7] produced reversible acute liver damage in dogs by intravenous injection

Subcutaneous $CrP^{32}O_4$ Rabbit Ears

Days		50uc	100uc	200uc	400uc
8	L&S	.0 %		.0 %	.0 %
	Node	0.3		.07	.08
14	L&S		.07 %	.0	.0
	Node		.0	.09	.16
37	L&S			.36	
	Node			.33	
61	L&S				1.72
	Node				2.34
85	L&S		4.6	1.3	
	Node		1.3	2.83	

Figure 2

and Au^{198} in doses delivered to the liver calculated at 47,000 to 67,000 rep per gram. Chronic damage was produced by doses of 65,000 to 85,000 rep. Koletsky and Gustafson [18] estimated that for Au^{198}, the threshold for liver damage in rats lies between 2,500 and 12,000 rep. They also found hepatic cell necrosis in rats which were given a single intravenous dose of 7 to 10 μc of radioactive colloidal chromic phosphate per gram of body weight.

SUBCUTANEOUS INJECTION OF RADIOACTIVE CHROMIC PHOSPHATE IN THE RABBIT EAR

Two series of six rabbits each were used in these experiments (Fig. 2). In one series 50 μc of Cr $P^{32}O_4$ was injected subcutaneously into the center of one ear of each of the six rabbits and 400 μc into the other ear. In the second series 100 μc was injected into one ear of each of the six rabbits and 200 μc into the other ear. All injections were in a volume of 0.2 ml. The animals were killed at intervals of 8, 14, 37, 61, and 85 days. Ten ears were assayed quantitatively with their respective nodes and liver and spleen. The opposite ears of these animals were used for histologic and gross examination along with the ears and organs of the remaining two animals. Twenty-four hours after injection the site of injection was red, and

on transillumination a grayish-green area of pigmentation was noted at the center of the injection site. The size of the induration and the density of gray pigmentation were in direct proportion to the microcurie dose. At the end of two weeks a small amount of crusting was noted in the center of each injected area, the size of which was in proportion to the microcurie dose and ranged from 2 mm. in diameter with the 50 μc dose to 1 cm. with the 400 μc dose. These crusted lesions progressed to dry gangrene. At the end of four weeks the gangrene had extended through the cartilage plate and into the epithelium on the opposite surface. Epilation of the overlying skin accompanied the extension of gangrene and extended approximately 2 mm. beyond the zone of erythema. From the fourth to the eighth week there was no lateral extension of the gangrene. The 400 μc dose resulted in a dry slough, leaving a cleanly punched-out hole in the ear. The addition of 500 viscosity units of hyaluronidase to 400 μc of chromic phosphate did not materially alter the area of distribution or the progressive destruction that followed the same dose of chromic phosphate without hyaluronidase. After the injection of 10 μc into a rabbit's ear, external counts over the area were begun immediately and were repeated at frequent intervals for a period of one month. The external counts gradually increased; and reached 10% two hours after injection. This increase was probably due to diffusion of the material beneath the skin. The external counts, corrected for decay, remained constant for four days, after which there was a sharp drop to half the original level, followed by a gradual drop to 10% of the original count at the end of four weeks.

The percentage of radioactivity accounted for by assay of the ear was 80% less than that calculated to be present on the basis of the amount injected. This decrease was due in part to loss by blistering and desquamation and in part to migration of particles from the site of injection.

The lymph nodes draining the ears were dissected and assayed for radioactivity in 10 animals, the remainder being used for gross and microscopic studies. The anterior and posterior auricular nodes in the 10 animals assayed contained measurable amounts of radioactivity, which remained essentially constant throughout the term of the experiment. In no animal did it exceed 1% of the administered dose. The amount of activity in the nodes decreased in proportion to the distance of the nodes from the site of injection. At the end of 8 and 14 days the anterior and posterior auricular nodes, (Fig. 2, Node 1) as well as the superior cervical nodes (Fig. 2, Nodes 2 and 3) were somewhat enlarged and dusky red. At the end of the 37th through the 85th day the nodes were pale tan. Microscopically, particles were identified in the nodes which gave a negative response to the Prussian blue test and were assumed to be chromic phosphate. Nodes removed at the end of the 8th and 14th days showed a marked increase in vascularity, with the sinusoids dilated and engorged. Germinal centers were inconspicuous and occasional polymorphonuclear leucocytes were noted. Lymph nodes removed at the end of the 37th through the 85th day were normal as compared with controls. Negligible radioactivity was found in the spleens and livers. Control animals received injections of nonradioactive chromic phosphate in the same dose suspended in 2% pectin solution. No changes were observed either grossly or microscopically, aside from the identification of particles presumed to be chromic phosphate at the site of injection.

Conclusions.—Radioactive chromic phosphate, 50 to 400 μc, injected into the subcutaneous tissue of the rabbit's ear produced a local area of dry gangrene at the

site of maximum radiation and an area of epilation which extended 2 mm. beyond the area of gangrene. The area of tissue damage was in proportion to the microcurie dose. Up to 1.4% of the total injected dose was picked up in the regional lymph nodes. The amount picked up by the lymph nodes decreased in proportion to the increasing distance of the nodes from the primary site of injection. Radioactive material did not reach the liver and spleen in appreciable amounts.

INTRAMUSCULAR INJECTION IN THE PECTORAL REGION

Twelve male rats each weighing approximately 150 gm. were used in this series of experiments; 200 μc of Cr $P^{32}O_4$ was injected into the right pectoral muscle of six animals and 100 μc into the same site in six other animals. Eight animals were killed at 13, 25, 39, and 53 days after injection. The remaining four animals were used for gross and microscopic studies. Initially the site of injection in the muscles was slightly indurated. An area of epilation was present over the site of injection in the animals killed on the 25th day. From the 25th day through the term of the experiment the area of induration underwent necrosis. Axillary and mediastinal

Intra muscular $CrP^{32}O_4$ Rat Pectoral

Day		100uc	200uc
13	L&S	1.00 %	2.00 %
	Node	0.04	1.00
25	L&S	0.38	4.40
	Node	6.00	7.00
39	L&S	1.08	0.02
	Node	6.00	1.00
53	L&S	0.02	0.02
	Node	0.04	2.00

Fig. 3.—Assay of liver and spleen (*L & S*) and axillary lymph nodes from eight animals, one at each time period and dose level.

nodes on the side of injection picked up a significant amount of radioactive material. The highest percentage of pickup in the axillary nodes and in the liver and spleen was 7% and 4.4%, respectively, of the initial dose 25 days after the animal had received 200 μc. The lowest node pickup was 0.04% 13 days after an injection of 100 μc. The lowest pickup in the liver and spleen was 0.02% 53 days after the injection of 200 μc. The assays are presented in Figure 3.

Two animals received injections of nonradioactive chromic phosphate equal in milligrams to the doses of radioactive material. There were no gross changes in the control animals when killed 53 days after injection. Animals were killed on the 7th, 14th, and 21st days for microscopic studies.

Microscopically, a progressive necrosis occurred at the site of injection the size of which was proportional to the microcurie dose and the time of exposure. The regional nodes showed evidence of an acute inflammatory reaction at 7 and 14 days which was not found at later dates. There were no demonstrable microscopic changes in the liver and spleen. All sections of radioactive tissue contained particles of brown material, assumed to be chromic phosphate.

Conclusions.—Radioactive chromic phosphate, 100 and 200 μc, injected into the pectoral muscle of rats caused local necrosis proportional to the microcurie dose and the time of exposure. Significant amounts, up to 7% of the total injected dose, were picked up by the regional lymph nodes, and up to 4% was picked up by the liver and spleen.

INJECTIONS OF RADIOACTIVE CHROMIC PHOSPHATE INTO THE TESTIS OF RATS

Twelve 150 gm. male rats were used in this series of experiments. Volumes of 0.2 cc. of 100 and 200 μc doses of Cr $P^{32}O_4$ were injected into the right testis of each of six animals.

Five animals were killed at 25, 39, and 59 days, and assays for radioactivity in the testis, regional nodes, liver, and spleen were done (Fig. 4). The remainder were used for gross and microscopic studies and were killed at 7, 14, 28, and 59 days. A control animal testis was injected with 2 mg. of nonradioactive chromic phosphate in a volume of 0.2 cc. This corresponded to the maximum amount of chromic phosphate injected into the experimental animals.

Interstitial $CrP^{32}O_4$ Rat Testicle

Days		100uc	200uc
25	L&S		4.4 %
	Node		1.0
39	L&S	.08 %	1.16
	Node	5.0	1.0
59	L&S	.02	.02
	Node	1.0	1.0

Fig. 4.—Assay of liver and spleen (*L & S*) and iliac, presacral, and periaortic nodes following injection of radioactive chromic phosphate into right testis, one animal at each dose level and time period.

Atrophy of the injected testis was first evident, by gross inspection, at the end of 7 days and was progressive until 28 days after injection, at which time the average weight of the injected testis was 0.25 gm., as compared with an average weight of 1.4 gm. for its uninjected mate. The surface of the injected testis was mottled grayish-yellow, and the parenchyma was for the most part necrotic. The common iliac nodes on the side of injection and the presacral and periaortic nodes from the animals killed seven days after injection were enlarged, dusky red, and pigmented grayish-green. These nodes were normal except for grayish-green discoloration in animals killed from the 14th through the 59th day.

Microscopically, the injected testis showed a center of necrosis of the parenchyma and complete absence of spermatogenesis of the tubules adjacent to the necrotic zone (Figs. 5 and 6). The area of necrosis was proportional to the microcurie dose and the time of exposure. The opposite testis was negative for radioactivity and was histologically normal (Figs. 7 and 8). The regional lymph nodes were inflamed in animals killed on the seventh day. This inflammation receded progres-

sively as the time interval increased between injection and the time the animals were killed. Particles assumed to be chromic phosphate were seen in these sections.

Assay of the regional lymph nodes for radioactivity revealed a maximum pickup of 5% 39 days after the injection of 100 μc. The highest pickup by the liver and spleen was 4.4% 25 days after injection of 200 μc. The entire carcass of one animal was dissolved in 2N NaOH solution after removal of the injected testis, the regional

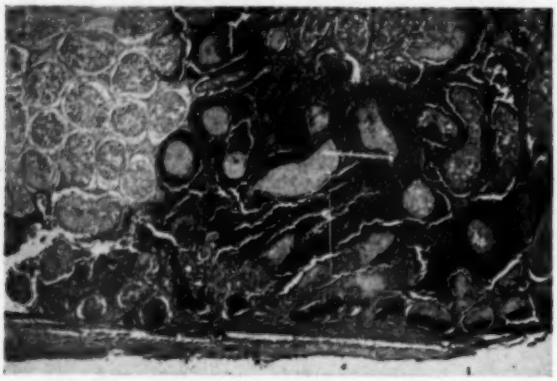

Fig. 5.—Low-power photomicrograph of testis 25 days after injection of 200 μc of radioactive chromic phosphate.

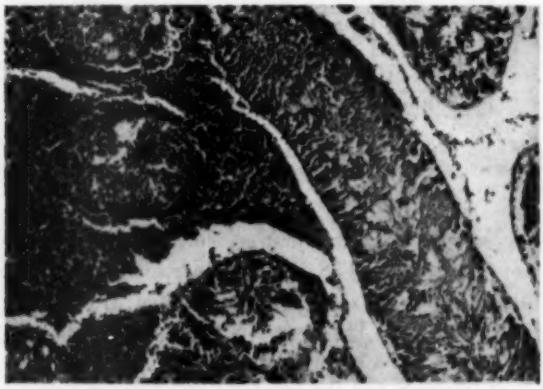

Fig. 6.—High magnification of injected testis.

lymph nodes, and the liver and spleen. Seven per cent of the total injected dose of 200 μc was recovered from this carcass.

Conclusions.—Radioactive chromic phosphate injected into the parenchyma of a rat testis produced central necrosis and a block of spermatogenesis proportional to the microcurie dose and the time of exposure without affecting the opposite testis.

Up to 5% of the injected dose of Cr $P^{32}O_4$ reached the iliac nodes on the side of injection and the presacral and periaortic nodes. Up to 4.4% of the injected dose reached the liver and spleen.

INFLUENCE OF PARTICLE SIZE AND SUSPENSION MEDIUM ON THE MIGRATION OF Cr $P^{32}O_4$

Because of variations in the migration of Cr $P^{32}O_4$ to the regional lymphatics, liver, and spleen it was assumed that the particle size and the physical characteristics of the suspension might have an influence on the migration. The following experiments were carried out to provide information on the influence of particle size and

Fig. 7.—Low magnification of uninjected mate of testis in Figure 5, showing absence of injury to germinal cells of tubules.

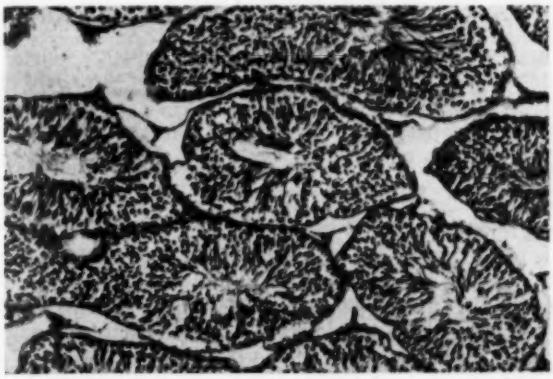

Fig. 8.—High magnification of uninjected mate of testis shown in Figure 5.

suspension medium on the migration of particles. Three combinations of Cr $P^{32}O_4$ were injected into the right testis of each of three pairs of 140 gm. rats in a constant volume of 0.2 ml. These volumes contained the same amount of carrier chromic phosphate (approximately 0.04 mg./0.2 ml.) but differed in specific activity.† The results, therefore, are given in percentage (Table 3).

† This material was being studied for the effect of particle size and vehicle on migration. Consequently, no attempt was made to use solutions of uniform specific activity. Activity was of importance only to assay the percentage migration.

Of the dose of 0.2 μ particles suspended in saline solution, 39.5% and 28.7% migrated to the liver and spleen. Of the 0.2 μ particles in pectin suspension, 13.7% and 15.8% migrated to the liver and spleen. From this one may conclude that particles in the range of 0.2 μ migrate from the site of injection in the testis to the liver and spleen. This mobility can be decreased by two-thirds when a protective coating, such as pectin, is provided for the particles.

Of the 0.2 μ particles in pectin, 13.7% and 15.8% migrated to the liver and spleen, while only 6.2% and 4.1% of the 2 to 4 μ particles migrated to the liver and spleen from the site of injection in the testis. The particle size markedly influenced the percentage of the injected dose which reached the liver and spleen.

Conclusions.—In a series of six animals, the smaller the particle size, the greater is the migration of chromic phosphate from the site of injection.

A 2% suspension of pectin decreased the mobility of chromic phosphate particles.

TABLE 3.—*Influence of Vehicle and Particle Size on Migration of Chromic Phosphate Injected into Rat Testis**

Percentage of Injected Dose Accounted for After 1 Wk. in	0.2 μ Particles in Saline		0.2 μ Particles in 2% Pectin		2-4 μ Particles in 2% Pectin	
	Rat # 1	Rat # 2	Rat # 3	Rat # 4	Rat # 5	Rat # 6
Liver	38.2	27.8	10.3	12.5	5.8	3.6
Spleen	1.3	0.9	3.4	3.3	0.4	0.5

* Each injection had a volume of 0.2 cc. containing approximately 0.04 mg. of Cr $P^{32}O_4$.

TABLE 4.—*One Millicurie of Cr $P^{32}O_4$ Injected into Right Pulmonary Hilus and Mediastinum*

% of Injected Dose Accounted for in	Dog No. 1 Right Lung Resected	Dog No. 2 Lungs Intact
Nodes, right hilus	0.2%	0.06%
Nodes, left hilus	2.7%	3.4%
Mediastinum	15.2%	14.5%
Diaphragm	6.9%	9.0%
Liver	15.3%	16.8%
Spleen	1.2%	1.3%

INJECTION OF Cr $P^{32}O_4$ INTRAPLEURALLY IN DOGS

Two 20 kg. dogs were used for these experiments. In one the right lung was resected and 1 mc. of Cr $P^{32}O_4$ was injected into the hilar region and mediastinum. In the second, the mediastinum was injected through a thoracotomy incision. Animals were killed after 24 days and the organs analyzed for Cr $P^{32}O_4$ and examined histologically.

The findings are listed in Table 4. There were no abnormal findings on histologic examination of the assayed organs. The average liver weight was 400 gm. with an average of 0.4 μc of Cr $P^{32}O_4$ per gram (wet weight), giving 351 rep of radiation per gram of liver.

COMMENT

Before using the colloidal suspension of radioactive material as a therapeutic agent in the treatment of cancer, one must determine whether the material will remain in situ after injection and, if not, how much will ultimately reach the liver,

spleen, and other organs. The most important factors influencing migration of locally injected chromic phosphate are the particle size and, to a lesser degree, the vehicle. If a suspension containing particles of 0.2 μ diameter in saline solution is injected interstitially, approximately one-third of the radioactive material reaches the liver and spleen. No more than 5% of particles 2.0 to 4.0 μ in diameter suspended in 2% pectin solution reaches the liver. In rats we found no changes in livers containing up to 28 μc per gram.

Koletsky and Gustafson [13] produced massive liver damage in rats by the intravenous injection of colloidal gold, Au^{198}. They found parenchymal atrophy, hepatic cell necrosis, and development of hepatic cell gigantism. The radiation provided to the liver in their experiments ranged in general from 24,000 to 42,000 rep, the smallest amount being 12,000 rep. The severity of the hepatic lesions was proportional to the dose. Hahn, Jackson, and Goldie [7] injected colloidal gold intravenously into dogs and caused acute damage of a partially reversible nature with 47,000 to 67,000 rep and chronic damage similar to a periportal cirrhosis with extensive fibrous tissue proliferation with 65,000 to 85,000 rep. The response of various animals is not uniform, nor can it be assumed that colloidal Cr $P^{32}O_4$ would necessarily affect a liver in a manner identical to that resulting from colloidal Au^{198}.

Wheeler, Jackson, and Hahn [22] injected 1 mc. of colloidal Au^{198} per kilogram of body weight into 14 dogs and reported no change in the thymol turbidity test.

A permissible dose of 3,000 rep to the human liver would seem well within tolerance in treating a patient with presumably incurable cancer. One microcurie of P^{32} with an average beta energy of 0.695 mev carried to complete decay furnishes 885 rep. The average human liver weighs 1,400 gm. Roughly, then, a human liver could retain 4,500 μc of colloidal radioactive chromic phosphate without immediate serious consequences. In our experiments on rats and rabbits, the maximum migration to the liver of colloidal Cr $P^{32}O_4$ with a particle size of 2.0 to 4.0 μ following interstitial injection was under 6% and was 16% following mediastinal injection in dogs. It is probably wise to assume that, under some circumstances, 20% of an injected dose may reach the liver. If the liver dose was limited to 4.5 mc. the permissible interstitial dose would be 22 mc.

In the preceding discussion we have assumed that damage to the liver is the only injury to be feared. Concomitant injury would occur also in the spleen, but this should not be of clinical importance. Only small amounts reach the bone marrow. We accounted for insignificant amounts of radioactive material in the urine after intravenous injection. Herve and Govaerts,[9] however, injected 3 mc. of radioactive chromic phosphate intravenously into five patients. In one patient the urinary excretion in five days totaled 8.05% of the dose. It was excreted as follows: 3.31% on the first day, 2.18% on the second day, 1.27% on the third day, 0.87% on the fourth day, and 0.42% on the fifth day. It is unlikely, then, that sufficient Cr $P^{32}O_4$ would be excreted or retained by the kidneys after interstitial injection to injure them. In one patient with a carcinomatous ascites, we gave an intraperitoneal injection of 10 mc. and obtained a total urinary output in 15 days of only 136 μc (Fig. 9). In another patient a 5 mc. intrapleural injection was followed by an inconsequential urinary output of radioactive material. Neukomm and associates [16] found a very small urinary excretion following injections into tumors of mice and, although they found some exchange of the phosphate radical into solubile PO_4, it was very small.

Judging from the effect when injection was made into the rabbit ear, one could predict that 50 μc of Cr $P^{32}O_4$ evenly distributed throughout 1 gm. of soft tissue would cause necrosis. If the total 50 μc remained in situ, the tissue would be exposed to 37,750 rep. Because of migration of some of the particles, it is probable that the actual dose delivered is in the neighborhood of 30,000 rep. This amount should be sufficient to destroy most tissues. The liquid, however, would have to be infiltrated uniformly throughout the tumor. Since such infiltration is rather unlikely, it would be best to assume that 100 μc per gram would be required to assure an adequate amount of destructive radiation in all parts of the tumor. Using 100 μc per gram of tissue to be treated and 22 mc. as the maximum permissible dose, theoretically one could infiltrate 200 gm. of tumor. The beta rays of P^{32} have a maximum range of only 8 mm. in tissue, and the effective therapeutic range is only 2 mm. We are able to destroy completely the rat's testis without affecting the neighboring testis. The problem of even infiltration is complicated by the fact that the structural peculiarities of many tumors will mitigate retention of injected fluid.

Up to 22 mc. of Cr $P^{32}O_4$ injected interstitially into a tumor would permit evaluation of this method with very little probability of untoward injury. Two sets of

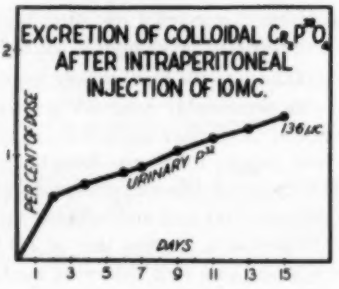

Figure 9

experiments have been performed by others in order to evaluate the effect of colloidal Cr $P^{32}O_4$ in malignant tumors in animals. Allen, Hempelmann, and Womack [1] injected colloidal Cr $P^{32}O_4$ interstitially into adenocarcinoma of the mamma in mice. Injections directly into the tumor caused death of the animals. The authors attributed the deaths to rapid entry of the Cr $P^{32}O_4$ into the blood stream via the vascular tumor tissue, since normal mice tolerated the same dose. After these observations the authors injected the chromic phosphate about the tumors rather than into them in a dose of 0.1 mc. for neoplasms less than 2 cm. in diameter. Tumors up to 2.5 cm. in diameter could be destroyed by this method of injection, but not those over 2.5 cm. in diameter. When the tumor was larger than one-fifth the body size, fatal "toxemia" often resulted. No distant parenchymatous damage was noted. Only traces of the colloid were found in the bone marrow, liver, and spleen, but there were considerable amounts in the regional lymph nodes, with definite damage resulting therefrom. No mention was made of the particle size. Yamashita and associates [24] injected about 0.8 μc of Cr $P^{32}O_4$ colloid per gram of tumor directly into or around sarcoma of mice. The rate of growth of the tumors slowed down, and in the more favorable cases actual shrinkage of the tumor was noted. The authors noted pickup of the

colloid by the regional lymph nodes. Since Hahn and his associates [6] introduced the concept of injecting radioactive material into a tumor, using Au^{198}, a number of investigators have explored the possibilities of this isotope. It has found some favor (Kerr and co-workers,[12] Sherman and associates,[20] and Soule [21]) in the treatment of carcinoma of the cervix and carcinoma of the prostate. Its short half-life necessitates the injection and handling of large amounts of the isotope, which in addition emits a gamma ray. The small particle size (approximately 0.03 μ) of its colloidal suspension favors its transport to the liver and spleen. Investigations being carried out at Oak Ridge, Tenn., on yttrium[90] hold promise that this isotope is preferable to both Au^{198} and Cr $P^{32}O_4$ for interstitial and intracavitary injection.

Jaffe [10] has made the first serious effort to evaluate the use of colloidal chromic phosphate clinically. He administered it intrapleurally to 30 patients with carcinomatous pleural effusion and obtained good results in about two-thirds of them. The usual dose was 5 mc., and he gave up to six doses to one patient. He also gave intraperitoneal injections to 20 patients with carcinomatous ascites, with improvement in 12 patients and no improvement in 8. The doses ranged from 5 to 15 mc. He also used the material interstitially in the treatment of carcinoma of the prostate in 28 patients. Doses of 6 to 40 mc. were injected into the prostate, the periprostatic tissue, and the seminal vesicles through the opened bladder. Needles were put in place throughout the area and a small quantity of the suspension injected into the area as the needle was withdrawn. The diluent solution consisted of 100 ml. of saline solution with 100 turbidity units of hyaluronidase and 1 ml. of 1 : 1,000 solution of epinephrine. Of the 28 patients, there was a decrease in the size of the cancer in 18.

Mumma [15] has reported on the treatment of a malignant mixed tumor of the tongue with radioactive chromic phosphate with disappearance of the tumor and no recurrence after three years.

We have found that, if the colloid is suspended in a 2% solution of pectin, it has a greater tendency to remain localized at the point of injection. This tendency is especially evident with smaller-particle suspensions but was not as clearly demonstrated in our few experiments with suspensions of larger particles. Under any circumstances vigorous shaking is necessary before the suspension is used. A 2% pectin solution is a safer vehicle for the chromic phosphate than isotonic sodium chloride solution. Herve and Closon [8] arrived at the same conclusion, using 10% gelatin solution as the vehicle. Tabern ‡ has recently made up a suspension in hypertonic glucose solution which is now available and which seems to possess the same holding qualities as pectin solution and in addition reduces the degree of clumping and settling.

The ideal volume of radioactive liquid to be injected into a tumor is problematical. We did not carry out precise experiments to answer this question. A single injection of 200 μc in 0.2 ml. will produce a larger area of necrosis than 50 μc in 0.2 ml., but it will produce a much larger area of necrosis if it is diluted and distributed more widely by means of multiple injections. Since one wishes to deposit about 100 μc throughout 1 gm. of tissue, this amount in 0.5 ml. of injectable liquid should give an adequate distribution. Clarke and LeRoy [5] recommended using a fluid volume of Au^{198} equal to one-third the volume of tissue to be treated and attempted to inject the quantity of Au^{198} to deliver 50,000 rep. This criterion is

‡ Tabern, D.: Personal communication to the authors.

probably equally applicable to the use of Cr $P^{32}O_4$. Sherman, Bonebrake, and Allen [20] found that colloidal gold when injected into humans subcutaneously soon spread over a large area. We injected 10 μc of Cr $P^{32}O_4$ in 0.2 ml. of fluid subcutaneously into a human and made radioautographs by placing a dental film over the area. The size and form of the autograph (even to minute details) remained unchanged for one week. The area measured about 2 sq. cm., and its edges were sharply defined. Two diametric methods of interstitial injection present themselves. The fluid may be injected forcibly into the tissue in the hope that it will spread effectively through the neoplasm. This is the method used by Kerr, Flocks, Elkins, and Culp [12] in injecting colloidal Au^{198} for carcinoma of the prostate. Or it may be distributed by the method of Jaffe, who places needles into the prostate in a precise geometric pattern and then injects a small quantity of the fluid into each area as he withdraws the needle. In a loosely constructed tumor or a fungating mass, this would be the only feasible method. When the material is injected locally, a significant amount reaches the regional lymph nodes. It would appear that the small particles pass through but the larger ones are retained in the lymph nodes. The nodes in the immediate vicinity may be destroyed, but in the majority the inflammatory process resolves. It cannot be assumed from this that a lymph node replaced by cancer would retain particles in transit from the point of local injection, though it seems likely that a node only partially replaced by a metastasis could accumulate enough ionizing radiation to destroy the malignant tissue.

SUMMARY AND CONCLUSIONS

Radioactive colloidal chromic phosphate injected intravenously into rabbits and rats is deposited to a large extent in the liver and spleen.

The mobility of particles injected subcutaneously into rabbits and interstitially into rats depends upon the size of the colloidal particles and the suspending media. About one-third of the dose reaches the liver when the particles are 0.2 μ in diameter and are suspended in saline.

If the colloidal substance is suspended in a 2% pectin solution, its mobility is significantly decreased.

Particles of 2 to 4 μ have significantly less mobility and are the size of choice if local effects are desired.

The possibility of treating tumors by local injection of radioactive colloidal chromic phosphate is discussed and its promise pointed out.

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METASTASES OF PRIMARY URINARY BLADDER CARCINOMA INVADING THE PROSTATE

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METASTASES from primary carcinomas of the urinary bladder are considered rare and are usually seen only late in the disease.[1] We have observed a number of such instances which we considered early cases and in which there were metastases to various organs, while in other, advanced cases the carcinoma was localized in the urinary bladder and there were no metastases. This investiga-

This study is based on autopsies on 61 primary carcinomas of the urinary bladder, of which 46 were in males and 15 were in females. The carcinoma was either

tion was undertaken to determine whether the presence of metastases was dependent

Table 1 is included to show the type and grade of malignancy, the location of the primary carcinoma, and the site of metastases of the 23 carcinomas of the urinary bladder which had invaded the prostate. Because of autopsy restrictions, a transitional cell carcinoma or a combination of transitional cell and squamous cell carcinoma. There was only one mucin-secreting adenocarcinoma.

Of the 46 cases in males, the primary carcinoma arose in the region of the trigone in 24 instances, in 10 at the dome of the bladder, and in 6 cases from the posterior wall, without involvement of the trigone. In five other cases the entire wall of the bladder was involved and the exact origin of the tumor could not be determined. In one case the tumor consisted of multiple papillary excrescences. The cases were graded according to Ash [2] on the basis of pleomorphism and mitoses of the cells, irrespective of their invasiveness. In 3 instances the carcinoma was Grade 1, in 20 Grade 2, in 11 Grade 3, and in 12 Grade 4. In 23 of the 46 males, the carcinoma had invaded the prostate. This was verified microscopically in every

upon the degree of malignancy or upon other factors.

instance: Seven of these carcinomas were Grade 2; 6, Grade 3, and 10, Grade 4.

complete examination of bones for metastases could not be performed. Only those few cases are listed in which osseous metastases were obvious in ribs, vertebrae, or pelvic bones.

From Table 1 it is evident that of 23 primary carcinomas of the urinary bladder invading the prostate, metastases were found in 21 instances in the following organs,

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and in this order of frequency: lymph nodes (pelvic, retroperitoneal, peritoneal, and distant), lung (including pleura), kidney, bone, and adrenal. In two cases there were no metastases. It is obvious that the occurrence of metastases did not depend upon the grade of malignancy as determined cytologically. Four carcinomas with malignancy Grade 2 produced metastases, while six, not listed in the Table, with malignancy Grades 3 and 4, did not produce any metastases. The commonest factor encountered in all our cases of primary urinary bladder carcinomas which had produced metastases was the secondary invasion of the prostate.

TABLE 1.—*Carcinoma of the Urinary Bladder Invading the Prostate*

Case No.	Type of Carcinoma	Grade	Location of Carcinoma	Location of Metastases
1.	Transitional cell	3	Posterior wall	Liver, lung, lymph nodes (pelvic)
2.	Transitional cell	4	Trigone	Liver, lung, adrenal, lymph nodes (pelvic, peritoneal, retroperitoneal, distant)
3.	Transitional cell	2	Trigone	Lung
4.	Squamous cell	2	Posterior wall	Lung
5.	Transitional cell	2	Trigone	Lung, kidney
6.	Transitional cell	4	Trigone	Bone, lymph nodes (pelvic, retroperitoneal)
7.	Transitional cell	4	Dome	Liver, adrenal
8.	Transitional cell	4	Posterior wall	Liver, lung, lymph nodes (pelvic, retroperitoneal)
9.	Transitional cell	3	Posterior wall	Lung, lymph nodes (distant)
10.	Transitional cell	4	Entire wall	Lung, diaphragm
11.	Transitional cell	3	Posterior wall	Liver, lung, kidney, bone, lymph nodes (pelvic)
12.	Squamous cell	3	Entire wall	Lung
13.	Transitional cell	4	Trigone	Liver, lymph nodes (pelvic, peritoneal, retroperitoneal)
14.	Transitional cell	2	Trigone	Liver, lymph nodes (pelvic)
15.	Transitional cell	3	Trigone	Lung, lymph nodes (pelvic)
16.	Transitional cell	4	Trigone	Liver, lung, kidney, bone, lymph nodes (retroperitoneal, distant)
17.	Transitional cell, squamous cell	4	Trigone	Lymph nodes (pelvic, peritoneal)
18.	Transitional cell, squamous cell	2	Entire wall	Lymph nodes (pelvic)
19.	Transitional cell	2	Dome	Lymph nodes (pelvic, peritoneal)
20.	Transitional cell	4	Trigone	Lymph nodes (pelvic, peritoneal, retroperitoneal)
21.	Transitional cell, squamous cell	4	Entire wall	Lymph nodes (peritoneal)
22.	Adeno	3	Trigone	.....
23.	Transitional cell	2	Dome	.....

Of 23 carcinomas which had not invaded the prostate only 5 produced metastases. Table 2 is included to give the pertinent data on these cases. It is of interest that the carcinoma in the first of these five cases had invaded a cystotomy tract and seminal vesicles; in the second case, a cystotomy tract, and in the third case, the rectus muscle. The fourth was microscopically found to be a diffusely infiltrating transitional cell carcinoma which had extended to the peritoneal and retroperitoneal regions. As demonstrated in Table 2, these four carcinomas had produced metastases. The fifth carcinoma, a transitional cell carcinoma Grade 2, arising in the posterior wall of the bladder above the trigone, had invaded neither the prostate nor any of the surrounding structures but had produced metastases to pelvic and retroperitoneal lymph nodes.

In summary, of 26 cases of primary carcinomas of the urinary bladder with metastases, the prostate was invaded in 21. Of the remaining five cases, in which the carcinoma did not invade the prostate but in which metastases were found, the primary carcinoma had invaded a cystotomy tract or had extended into surrounding structures in four. In other words, out of a total of 26 cases of primary carcinoma of the urinary bladder with metastases, the prostate or the surrounding tissues were invaded in 25.

Of the cases of primary carcinomas of the female urinary bladder, metastases were found in six. In one of these there was invasion of the parametrium and of the cervix uteri. There was no gross invasion of adjacent structures in the other five cases. Unfortunately, no sections were taken from the cervix and parametrium for microscopic study. However, in a previous study of four cases of primary carcinomas of the urinary bladder in the female with metastases, one of us (O. S.) [3] found microscopic invasion of the parametrium in all. Because of the insufficient material available for study in these five cases, no conclusions can be drawn.

TABLE 2.—*Carcinoma of the Urinary Bladder Not Invading the Prostate*

Case No.	Type of Carcinoma	Grade	Location of Carcinoma in Urinary Bladder	Location of Metastases	Comments
1.	Transitional cell	2	Trigone	Retroperitoneal lymph nodes	Invading cystotomy tract and seminal vesicles
2.	Transitional cell	2	Dome	Liver, lung, peritoneal lymph nodes	Invading cystotomy tract
3.	Transitional cell	2	Dome	Liver, bone, peritoneal lymph nodes	Invading rectus muscle
4.	Transitional cell	3	Trigone	Peritoneal lymph nodes	Extension to retroperitoneal space
5.	Transitional cell	2	Posterior wall	Retroperitoneal and pelvic lymph nodes	.....

While metastases from primary carcinomas of the prostate are common, those from primary carcinomas of the urinary bladder are usually regarded as being rare. This may be because death occurs before metastases develop.[1] We might mention in this connection that most of our patients died af ascending pyelitis and pyelonephritis. There are only a few reports of individual cases or groups of cases demonstrating metastases from urinary bladder carcinomas. Some of these reports stressed that late carcinomas of the urinary bladder penetrate the wall and may extend to the peritoneum with the production of metastases. Unfortunately, many of these studies simply state "penetration" of the tumor or "extension" to the serosa but do not mention whether or not the prostate was invaded by tumor. In a previous study one of us [3] had remarked on the importance of the examination of the prostate in the evaluation and prognosis of bladder carcinomas.

In our study we have found that of 26 cases of primary urinary bladder carcinoma in the male that had produced metastases, the prostate was invaded in 21. It seemed obvious that the occurrence of metastases did not depend upon morphologic grades of malignancy. The high percentage of our cases in which there were metastases and also invasion of the prostate is significant, and is not a coincidence.

Walther,[4] in his monograph on carcinoma metastases, stated that urinary bladder carcinomas may invade the adjacent connective tissue of the pelvis, but did

not mention invasion of the prostate. Kretschmer [5] reported eight patients with carcinoma of the urinary bladder with metastases, of whom five died. Unfortunately, on only one of these was an autopsy performed, and no mention is made of whether or not the prostate was invaded. Thus the possibility cannot be ruled out that the prostate was invaded or that there might not have been also another carcinoma present, possibly in the lung, which may have been responsible for the metastasis. The series of cases reported by von Rütte [6] is interesting, since he reported metastases in 55 of 121 cases of carcinoma of the urinary bladder. However, the report is rather sketchy, and, again, there is no statement as to whether the prostate was involved. As a matter of fact, it is interesting that his figures for metastases, amounting to 45%, compare very well with our percentage (45.5) of cases of carcinoma with involvement of the prostate which have produced metastasis.

It is well known that primary carcinomas of the prostate often produce metastases very early and that not rarely such a metastasis may be recognized clinically much sooner than the primary tumor. Pulmonary metastases, common in prostatic carcinomas, were found in 12 of our cases in which there was secondary prostatic involvement. While it is true that metstases of prostatic carcinomas are commonly located in the osseous system, obvious bone metastases were encountered in only three of our cases of bladder carcinomas. However, as mentioned before, no systematic search for osseous metastases could be undertaken because of autopsy restrictions. It might very well be that invasion of the prostate per se signifies greater malignant tendency than can be surmised from morphologic grading and is a much more important factor in estimating the degree of malignancy than morphologic and cytologic characteristics of tumor cells. However, it is intriguing to speculate whether perhaps the carcinoma of the urinary bladder, when it invades the prostate, in some respects behaves biologically more like a primary carcinoma of the prostate. Unfortunately, no phosphatase determinations had been performed in these cases.

It might be argued that invasion alone implies a higher degree of malignancy, as stressed again recently by McDonald and Thompson.[7] This is perfectly true, although, from the foregoing, it seems that if the prostate is invaded, distant metastases are much more likely to occur than if other adjacent structures are invaded. As mentioned before, among the five carcinomas which had not invaded the prostate but had produced metstasis, two had invaded a cystotomy tract, one the rectus muscle, and one the retroperitoneal space.

It might also be mentioned that in some of the sections taken from the prostate showing invasion by bladder carcinoma, tumor cells were noted in the perineural lymphatics. This has been found commonly in primary prostatic carcinomas, as reported by Moore [8] and by Warren and associates,[9] who stressed that this finding is very characteristic of primary prostatic carcinomas.

There are many cases on record of so-called bizarre and unexpected metastases [10] from various primary carcinomas. From our study, unusual metastases may perhaps be explained not just by the location and type of the primary carcinoma but also by the secondary structures invaded. It appears that a primary carcinoma of the urinary bladder upon invasion of the prostate behaves, as far as metastases are concerned, like a primary carcinoma of the prostate.

SUMMARY

Primary carcinomas of the male urinary bladder were studied in 46 instances. The morphologic degree of malignancy of these tumors varied from Grade 1 to Grade 4. Metastases occurred in 26 of these cases, in 21 of which the tumor had invaded the prostate. The occurrence of metastases could not be predicted from the degree of malignancy of the primary tumor.

In 23 cases the prostate was directly invaded by the urinary bladder carcinoma. Of these 23 cases, 16 showed diffuse metastases resembling those usually produced by primary carcinoma of the prostate.

The degree of malignancy of the carcinomas which had invaded the prostate and produced metastases varied from Grade 2 to Grade 4. It seems that it is not so much the grade of malignancy which is responsible for the metastases as it is the invasion of the prostate.

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PERIARTERITIS NODOSA IN A WEEK-OLD INFANT

Report of a Case with Necropsy

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IN THE present case, widespread, acute lesions of periarteritis nodosa were found at necropsy in an infant dying on the seventh day after birth. Other important findings included an aneurysm, with thrombosis, of the ductus arteriosus, that had given rise to renal embolism and subsequent massive but incomplete renal infarction. The necrotizing vascular lesions closely simulated, histologically, the various stages of periarteritis nodosa as produced experimentally in rats, by Zeek, Smith, and Weeter,[1] within seven days after procedures that caused renal ischemia and hypertension.

REPORT OF CASE

Clinical Course.—The infant was the first child of healthy parents aged 21 and 23 years. The gestation and delivery at term, in another hospital, were normal. Vitamin K, 5 mg., was administered at birth. Breast and supplemental feedings were given from the first day. On the second day a rash was noted, and a 5% sulfonamide ointment was prescribed. Circumcision, on the fourth day, was followed by normal healing. At the time of discharge, on the fifth day, the child appeared normal except for slight hoarseness. A diagnosis of thrush was made. Vitamins C and D were added to the diet.

On the morning of the seventh day slight stuffiness of the nose was noted, and the infant passed several loose, green stools. There had been no known exposure to any contagious or other disease. That afternoon he vomited material containing flecks of blood. Following this episode, the child's color seemed abnormal, and his respirations became very rapid. He was brought promptly to the Children's Hospital.

Physical examination revealed a well-developed and well-nourished infant in marked respiratory distress. The cool, dry skin appeared mottled and cyanotic, but there was no evidence of a rash or petechiae. The rectal temperature was 99 F. The anterior fontanelle was open and flat. The ears and eyes were normal, but the fundi were not examined. The nares dilated with respirations. Patches of a necrotic pseudomembrane covered the tongue and buccal surfaces. The pharyngeal mucosa was congested. The neck was supple. The superficial lymph nodes were not palpable.

The heart sounds were of good quality, with no murmurs, but the apical beat was 180 per minute. Labored respirations were accompanied by grunting sounds and by intercostal and infracostal retractions. The inspiratory phase was unhampered, but the expiratory phase was labored. There was poor transmission of the breath sounds, and fine, moist rales were audible bilaterally with each inspiration. The rate varied from 60 to 80 per minute.

The edge of the liver was palpable 3 cm. below the right costal margin, the abdominal wall being soft and flaccid. There was no sign of inflammation in the dry stump of the umbilical cord. The glans of the penis was cyanotic, but the circumcision wound manifested normal healing. The genitalia were otherwise normal. No abnormalities of the extremities were found.

From the Departments of Pathology, Children's Hospital, and Cincinnati General Hospital, and from the Department of Pathology, University of Cincinnati College of Medicine.

Laboratory Data.—Hemoglobin, 8.0 gm. per 100 cc. Red blood cells, 2,200,000 per cubic millimeter; white blood cell count, 11,500 with 75% neutrophiles, 24% lymphocytes, and 1% monocytes. Chemical examination of the blood revealed nonprotein nitrogen, 43 mg. per 100 cc.; serum pH, 7.19; carbon dioxide, 11.6 mEq. per liter; serum chlorides, 99.2 mEq. per liter; carbon dioxide tension, 28 mm. Culture of the blood yielded no growth. Hemolytic Staphylococcus aureus, Neisseria sicca, and hemolytic Staphylococcus citreus were recovered from the nasopharynx on culture.

Roentgenographic examination of the chest revealed both lungs filled with material of water density, suggesting massive inflammation or extensive aspiration pneumonia. The cardiac shadow was not identified because of the pulmonary density.

Course in Hospital.—The child was promptly treated with 5% dextrose and isotonic saline given intravenously, 40,000 units of penicillin, 40 mg. of streptomycin, and 5 mg. of vitamin K. He was placed in an incubator with continuous oxygen. The cyanosis lessened slightly, but the skin remained mottled. Respirations became more labored, and the heart rate increased. Tracheal suction and, later, bronchoscopy with suction yielded hemorrhagic fluid from the bronchi. Unfortunately, no blood pressure reading was recorded. The child died 3 hours after admission to the hospital and in less than 24 hours after the onset of symptoms.

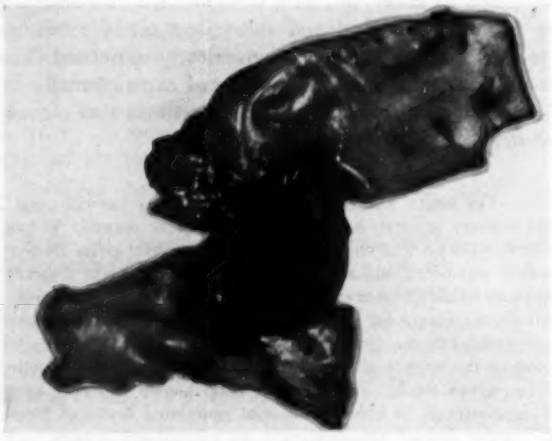

Fig. 1.—Aneurysm, with thrombosis, of the ductus arteriosus.

Necropsy.—The well-nourished body weighed 3,700 gm. and measured 56 cm. from crown to heel. The lips, tongue, and nail beds were moderately cyanotic. Bloody fluid filled the nose, mouth, and entire tracheobronchial tree. The voluminous lungs were dark-red and wet. The pulmonary vessels appeared normal. The pulmonary changes were interpreted microscopically as early aspiration pneumonia.

The heart and aorta appeared normal grossly. About two-thirds of the ductus arteriosus, at the aortic end, was dilated to form an aneurysm 10 by 7 by 7 mm. in diameters (Figure 1). It was filled with a dark-red, slightly adherent thrombus. The pulmonary end of the ductus arteriosus appeared normal and communicated through a 1 mm. orifice with the pulmonary artery. The ostium at the aortic end measured 2 mm. in diameter.

The mucosa of the esophagus was covered by a thick, shaggy layer of inflammatory exudate, which was lightly attached to the mucosal surface. The stomach and duodenum appeared normal. The mucosa of the slightly dilated small intestine was sparsely mottled with small submucosal hemorrhages, but there was no ulceration. The colon appeared normal, and no gross changes were noted in the mesentery or in any of the splanchnic vessels.

About two-thirds of the right kidney, including the superior and inferior poles, presented confluent areas of swelling and discoloration characteristic of acute renal infarction. The main

renal artery was not occluded, but one of its major branches near the renal pelvis contained a mass of thrombotic material lightly attached to the normal-appearing wall of the vessel just proximal to its bifurcation. In the infarcted regions the pyramids were very hemorrhagic, while the cortical portions had yellow ischemic centers and hyperemic margins.

The left kidney appeared normal grossly. No significant lesions were found in any of the other viscera. The brain was not removed. Cultures of lung and spinal fluid yielded no growth.

Microscopic Examination.—Serial sections through the ductus arteriosus revealed beginning organization in the attached thrombus, which overlay areas of acellular, noninflammatory mucoid degeneration of the intima and inner media (Fig. 2). The outer media and adventitia contained many small blood vessels, and in a few sections a sparse sprinkling of leucocytes was seen. The degenerative

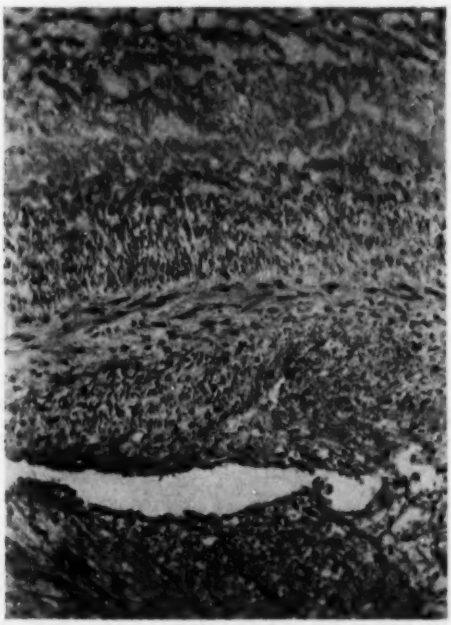

Fig. 2.—Section through the ductus arteriosus. Mucoid degeneration of the media near the top of the figure and laminated thrombus at the bottom, attached at the right. × 120.

changes were not confined to the wall of the aneurysm but were found also in sections from the pulmonary end of the ductus arteriosus. No bacteria were found, and there was no fibrinoid necrosis or other evidence of periarteritis nodosa in this structure.

Likewise, the obstructed renal artery presented no evidence of periarteritis nodosa. The wall beneath the clot appeared normal microscopically. Frank necrosis and marginal zones of beginning neutrophilic reaction characterized the renal infarcts. Although there was no infarction of the left kidney, microscopic sections revealed many microemboli in scattered cortical arterioles and glomerular capillaries, all very recent in origin, since there was no necrosis or inflammatory reaction.

The most interesting findings in the kidneys were characteristic early lesions of periarteritis nodosa, involving small arteries near both renal pelves and a few corticomedullary branches in the left kidney and in the noninfarcted portions of the right kidney. The earliest lesions were found only at sites of branching or bifurcation and consisted of fibroblastic and histiocytic proliferation in the adventitia and at the medioadventitial junctions (Fig. 3). Frequently small foci of fibrinoid necrosis, characterized by eosinophilic smudging and karyorrhexis, could be seen in the media in or near the crotch of a bifurcation, when that part of an involved

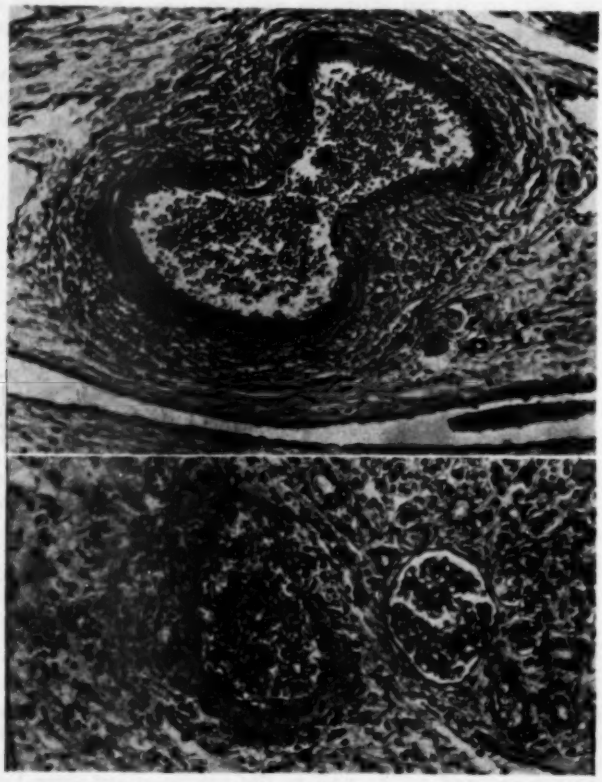

Fig. 3.—Periarteritis nodosa, early lesion, proliferative stage, in a bifurcating artery near the renal pelvis. × 120.

Fig. 4.—Periarteritis nodosa, early exudative stage. Necrosis of the media. The artery is situated near the uninfarcted portion of the right kidney. × 120.

vessel was included in the section. There was a peculiar tendency for the proliferating fibroblasts in the adventitia to become oriented in a parallel or end-to-end relationship with the cells of the media, simulating a radiation effect, thus differentiating the early proliferative lesions of periarteritis nodosa from other, commoner types of perivascular fibrosis.

In a few renal arteries the lesions had progressed to extensive fibrinoid necrosis, accompanied by the exudation of fluid and inflammatory cells of various types from

the blood stream (Fig. 4). None of the renal lesions had progressed to thrombosis, aneurysm formation, healing, or vascular occlusion. Interval sections through regions supplied by the obstructed renal artery revealed no lesions of periarteritis nodosa in any branch distal to the site of obstruction, although typical exudative

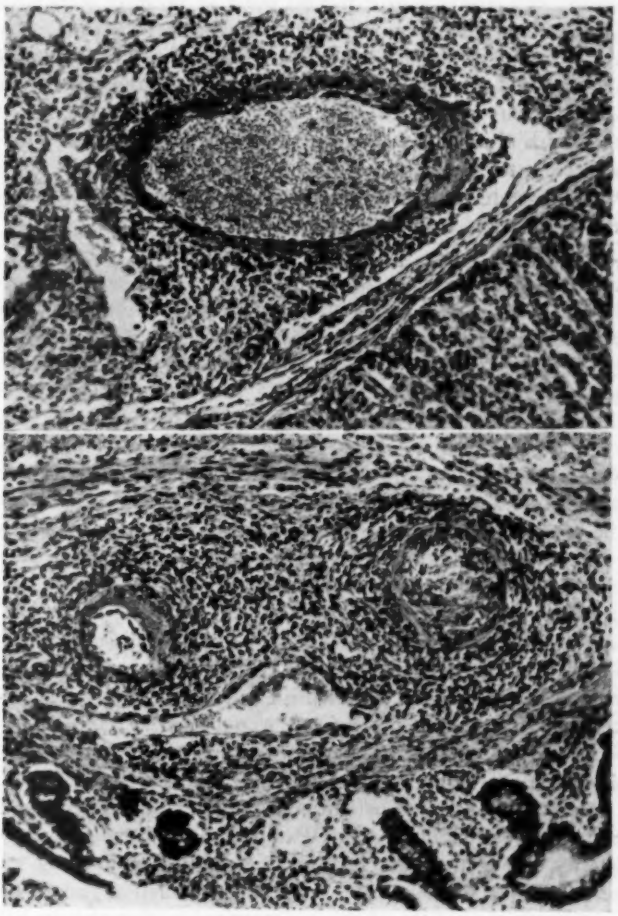

Fig. 5.—Periarteritis nodosa, exudative stage, in an artery near the adrenal. × 120.

Fig. 6.—Periarteritis nodosa, full-blown exudative stage with beginning thrombosis, in arteries just distal to the bifurcation of a mesenteric artery near the site of mesenteric attachment. × 120.

lesions were found in a few branches of a nonobstructed artery supplying the central, noninfarcted portion of the right kidney (Fig. 4).

Similar exudative lesions of periarteritis nodosa were found in the ileum, periadrenal tissues (Fig. 5), and testis. The oldest lesions were in the ileum, where the branches of small mesenteric arteries, just distal to the bifurcations, at sites of

mesenteric attachment, presented exudative lesions that had progressed to beginning thrombosis (Fig. 6). No complete occlusion was found, and the intestine was not infarcted.

Microscopic sections of the pancreas, bladder, and trachea contained early proliferative lesions of periarteritis nodosa with only slight necrosis (often found only by serial sectioning). Proliferative lesions with no necrosis were present in the esophagus, prostate, striated muscle, heart, duodenum, jejunum, and lymphoid structures. No lesions of periarteritis nodosa were found in the lungs, although many vessels had collars of fibrous tissue, a common finding in the lungs of the newborn. The fibrous tissue was well collagenized. Orientation of fibroblasts, fibrinoid necrosis, and inflammatory exudation were absent, thereby differentiating this condition from the proliferation stage of periarteritis nodosa.

The only other noteworthy microscopic findings were acute esophagitis (many bacteria and Candida present) and low-grade enteritis.

Diagnoses.—Aspiration pneumonia (immediate cause of death); aneurysm, with thrombosis, of the ductus arteriosus; renal embolism; massive but incomplete infarction of the right kidney; microembolism in the left kidney; acute periarteritis nodosa, widespread but not in the lungs, involving many muscular-type arteries, the oldest lesions being in the ileum; acute thrush; esophagitis; low-grade enteritis.

COMMENT

Many investigators, cited in a recent review by Zeek,[2] have noted the occurrence of lesions closely resembling the Kussmaul and Maier type of periarteritis nodosa in the course of various experimental procedures that cause renal ischemia and hypertension in rats and other laboratory animals. Certain differences have been described between these lesions and the necrotizing angiitis associated with hypersensitivity to sulfonamides and other antigens in man.[1] Some investigators believe that both conditions occur in man and have described distinctive features in regard to the morphology and distribution of lesions, and, likewise, important differences in the clinical course and symptomatology [3] that aid in differentiating those two conditions both clinically and pathologically. Nevertheless, other investigators consider the necrotizing arteritis of hypertensive rats as a reaction peculiar to the rat and not occurring in man. The present case would seem to refute this concept.

In the present case the question of hypersensitivity might be raised, since a sulfonamide ointment was prescribed for a rash five days before death. Clinically there was no evidence of hypersensitivity, since the rash preceded the therapy and disappeared promptly after the therapy. Pathologically, the involvement of muscular-type arteries rather than the intrinsic arterioles, venules, and capillaries of viscera, the absence of lesions in the pulmonary circulation and in the follicular arterioles of the spleen, and the absence of necrotizing glomerulonephritis sharply differentiate the vascular disease in this case from that found in the 10 cases of hypersensitivity angiitis included in a recent report by Knowles, Zeek, and Blankenhorn.[3] In 7 of their 10 cases the onset of hypersensitivity symptomatology was directly related to sulfonamide therapy.

In 1946 Loomis [4] produced hypertension and necrotizing arteritis in rats by infarcting varying proportions of one or both kidneys. She found that the incidence of these two conditions was inversely related to the amount of normal renal tissue left rather than being entirely dependent upon the amount infarcted. She suggested a sensitization to some by-product of necrotic renal tissue as the possible cause of

the vascular lesions. The work of many investigators has proved that kidneys need not be made ischemic to the degree of infarction in order for hypertension and necrotizing arteritis to occur.

Although hypertension often occurs in experimental animals and in man without necrotizing angiitis, the Kussmaul and Maier type of periarteritis nodosa is rarely found in the absence of hypertension. The latter, however, is very difficult to prove, and it is conceivable that necrotizing vascular lesions may be initiated by an acute episode of rapidly developing hypertension of short duration. Such an episode could easily be missed clinically. On the other hand, the frequent association of these two conditions may depend upon some factor which is common to the pathogenesis of each. An intriguing question is whether vascular lesions, identical in every respect with those found in the present case, can develop after bilateral nephrectomy or in association with neurogenic hypertension when the kidneys are entirely normal.

SUMMARY

A case of widespread, acute periarteritis nodosa in a week-old infant is recorded. The lesions apparently developed after birth and followed massive but incomplete renal infarction, the latter being caused by embolism from a thrombus in an aneurysm of the ductus arteriosus. The vascular lesions in this case appeared to be identical in morphology, distribution, and stages of lesions represented with the necrotizing arteritis produced experimentally in rats by many investigators who have employed procedures which caused renal ischemia and rapid rise in blood pressure. The various stages of the lesions in this case have been produced in rats within a similar period of seven days. This case refutes the concept that the rat type of periarteritis nodosa does not occur in man.

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Cincinnati General Hospital (Dr. Zeek).

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ACQUISITION OF TOPICAL UNRESPONSIVENESS TO TISSUE INJURY

III. Topical Thermal Preirritation Preventing Hemolymph Node Formation After Burning

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TISSUES subjected to inflammatory irritation can acquire topical unresponsiveness to a subsequent injurious stimulation. In previous reports, topical tissue unresponsiveness to blood-borne irritants has been demonstrated in the rat paw preirritated by various inflammatory stimuli [1]; further, it has been shown that a mild thermal preirritation of rat and rabbit tissues induces topical unresponsiveness to a subsequent stronger thermal irritation leading to inflammation and necrosis in normal tissues.[2]

In the investigation reported here, an attempt was made to obtain further information on the mechanisms underlying acquired topical tissue unresponsiveness. In this connection, the possibility was considered that in preirritated tissues the formation and/or liberation of intermediary inflammatory substances * may become impaired, and that this may account for the decreased topical reaction to subsequent injurious stimulation. As toxic substances had been extracted from burned tissues (data and review of earlier work by Cullumbine [10]) and had also been found in the lymph draining burned areas,† it was thought that the macroscopic and microscopic appearance of regional lymph nodes after thermal injury might serve as an indicator of the occurrence of intermediary toxic products in the tributary tissues.

The experiments were performed on normal and thermally preirritated rat paws. Throughout the experiments, thermal irritation was produced by "poikilothermal heating" (immersion of the paws in water under compression of the blood vessels), which permitted an equal and homogenous iritation of normal and preirritated tissues.[13] The perviousness of the draining lymph vessels after heating normal and thermally preirritated tissues was tested by injecting trypan blue or India ink into the heated paws and by recording subsequently the appearance of the color particles in the regional lymph nodes.

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This study was performed with the aid of a Fellowship granted by the Canadian Life Insurance Officers Association.

* Widely discussed by Menkin, references 3 and 4, Duthie and Chain,[5] Cullumbine and Rydon,[6] Spector,[7] Rosen and Levenson,[8] and Westphal.[9]

† References 11 and 12.

METHODS

Thirty male Sprague-Dawley rats weighing 100 to 120 gm. were used.

"Poikilothermal Heating."—For preirritation, the right hindpaw, under compression of the abdominal aorta with a rubber tube, was immersed in 48 C. water for 2 minutes 15 seconds. Immediately afterward, the compression was released. The degree of the ensuing inflammation, measured by the increase in the dorsoplantar diameter of the paw at the level of the metatarsus, was mild (maximum increase below 50%). For the stronger heat irritation two days later, when the diameter and the appearance of the right paw was almost back to normal, both paws under aorta compression were placed in 49 C. water for two minutes. Measurements of the tissue temperature in the paws during the second heating had revealed [13] that the duration and the degree of temperature increase in the right paw were not less than in the left paw, even when the right, preirritated, paw still showed a slightly increased diameter. In general, a faster rise in temperature was observed in the right, preirritated, paw, possibly due to a higher water content of the tissues after the first inflammation and a resulting increase in heat conductivity (Henriques and Moritz ‡). Since the paws were immersed only up to the ankle, the popliteal lymph nodes were not directly irritated.

In two additional experiments, India ink (0.1 cc. of a 0.1% dilution of Waterman's India ink) and trypan blue (0.1 cc. of a 0.3% solution) were injected beneath the plantar aponeurosis of both paws immediately after heating, the conditions of which will be indicated separately.

Histology.—Immediately after death, all specimens were fixed in susa solution. After fixation, the tissues were embedded in paraffin, sectioned at 6μ, and stained with hematoxylin and eosin.

RESULTS

Prevention of Inflammation and of Hemolymph Node Formation.—Figure 1 shows the degree of inflammation after strong thermal irritation of both hindpaws in a group of eight rats. The right paw was subjected to a mild heat irritation two days prior to the second heating. As can be seen, the preirritated paw showed considerably less inflammation than the normal left paw. After the animals were killed by a blow on the head and exsanguination 14 hours after the second heating, naked eye examination of the popliteal lymph nodes revealed marked marginal hemorrhages in the left side but only a few scattered hemorrhages and sometimes none in the right. The protection against inflammation in the right paw and the absence of hemolymph node formation in the draining lymph node are shown in Figure 2. As measured by an arbitrary scale of 1 to 3 +, the mean and standard error of the degree of hemorrhages in the left popliteal lymph nodes of the eight rats were 1.83 ± 0.46, and in the right 0.44 ± 0.25.

In 10 other rats, it was found that after "poikilothermal heating" of normal paws (49 C. for two minutes), the hemolymph node formation in the popliteal lymph nodes appeared, beginning with petechial hemorrhages, in less than 10 minutes,

‡ Reference 14. In the experiments of these authors the increased thermal conductivity of edematous dermis did not result in a faster rise in subcutaneous tissue temperature if heat was applied on the skin surface without stopping local blood circulation.

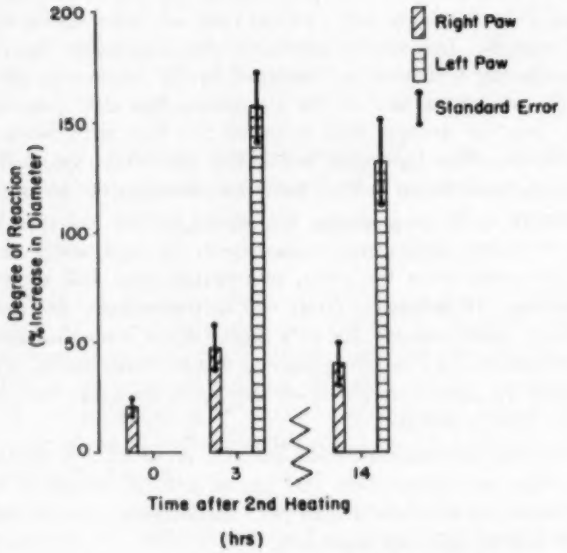

Fig. 1.—Degree of inflammatory reaction after burning of both hindpaws (two minutes, 49 C., aorta compression). Right paw preheated. Note the much smaller reaction of the preirritated right paw (group of eight rats).

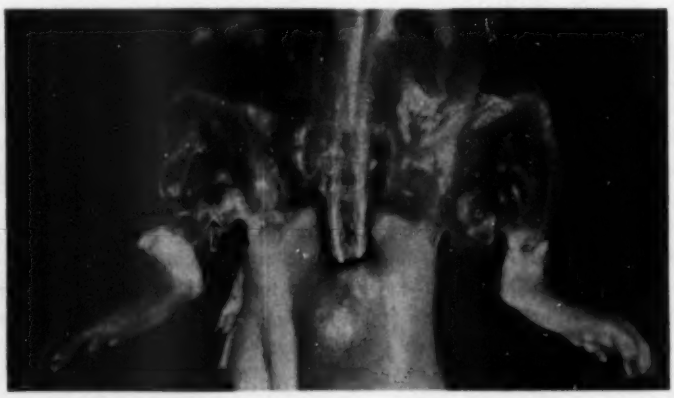

Fig. 2.—Prevention of hemolymph node formation after burning by thermal preirritation. Rat killed 14 hours after thermal irritation of both hindpaws. Right paw preheated. In this rat, the diameter of the two paws were identical at the time of the second heating. Massive swelling of the left paw and hemolymph node transformation of the left popliteal lymph node. Much smaller inflammatory reaction of the preirritated right paw and normal appearance of the draining lymph node.

thus preceding the anatomic changes in the paws in which the maximal inflammation was reached only 3 to 4 hours later. Two to three days after heating, the macroscopic appearance of the lymph nodes became normal again.

Perviousness of Lymph Vessels and Architecture of Lymph Nodes After Burning.—When trypan blue was injected into both hindpaws (six rats) immediately after heating of the right paw (49 C. for two minutes) and the rats were killed five to six minutes later, the left popliteal lymph nodes were intensely blue colored, whereas in the right nodes petechial hemorrhages had occurred. No color particles appeared in the right nodes. Such a fixation of particles in inflamed tissues § makes it unlikely that particles like erythrocytes could have escaped from the heated tissues by way of the lymph vessels. Therefore, the petechial accumulations of erythrocytes in the lymph nodes shortly after burning of their tributary tissues can be considered true hemorrhages.

When India ink was injected into both hindpaws, two days after thermal preirritation of the right paw (48 C. for 2 minutes 15 seconds) and immediately after the subsequent heating of both paws (49 C. for 2 minutes), this did not affect the

Histologic Changes in the Regional Lymph Nodes, 2 Days 15 Hours After Thermal Preirritation of the Right Hind Paw (48 C. for 2 Minutes 15 Seconds) and 15 Hours After Heat Irritation of Both Hindpaws (49 C. for 2 Minutes), Immediately After Which Both Paws Were Injected with India Ink

Degree *	Left Iliac Lymph Node						Right Iliac Lymph Node						Left Popliteal Lymph Node						Right Popliteal Lymph Node					
Rat no.	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Hemorrhage	1	0	0	3	2	1	0	0	0	0	0	0	3	3	3	2	2	3	½	0	0	0	0	2
Depletion of lymphocytes	1	1	2	3	½	2	0	½	0	½	0	0	1	2	2	1	3	3	½	0	0	0	0	½
Edema	0	1	1	3	2	2	2	1	0	1	1	1	2	2	½	1	3	1	1	0	0	1	1	2
Ink particles accumulation	0	0	0	0	0	0	3	1	1	1	½	1	0	0	0	0	0	0	½	1	1	1	1	½

* One plus to three plus.

inflammatory reaction of the left normal paw or the failure to react of the preheated right paw. The Table shows the degrees of hemorrhage, depletion of lymphocytes in the center of the lymph nodes, edema formation, and accumulation of ink particles in the iliac and popliteal lymph nodes of both sides, as found in specimens taken 15 hours after the second heating (group of six rats). Extensive hemorrhage, edema, and depletion of lymphocytes appeared in the left popliteal and iliac nodes, whereas in the right side only a slight edema formation, occasional spots where lymphocytes had been replaced by reticular cells, and occasional hemorrhages were observed. Some of the right lymph nodes did not change their normal architecture after the second heating. Ink particles were found in the right but not in the left nodes, indicating that no particles had escaped from the severely inflamed paw. On the other hand, the fact that the particles had left, apparently normally, the preirritated protected paw excludes the possibility that a mechanical barrier could have prevented the protected right nodes from being reached by toxic products carried by the lymph flow from the burned area, should such products have been liberated there. In Figures 3 to 6, histologic sections of the right and left popliteal lymph

§ References 15 and 16.

nodes from one rat of this group are shown 15 hours after thermal injury of the paws. Hemolymph node formation had occurred in the left node (Figs. 3 and 5), whereas the right node draining the thermally preirritated paw had retained the

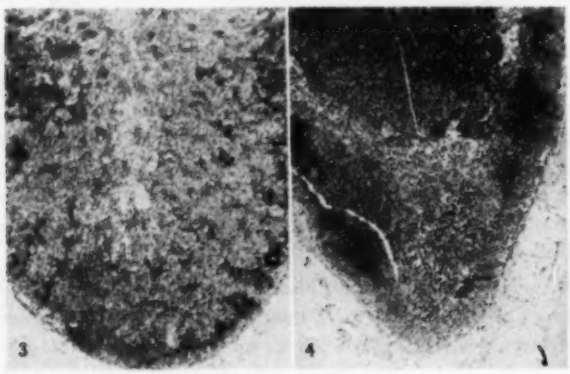

Fig. 3.—Popliteal lymph node draining the left hindpaw 15 hours after thermal injury of the paw. Note central portion depleted of lymphocytes. The center consists mainly of reticular cells, a few macrophages, plasma cells, and scattered smaller lymphocytes and shows masses of erythrocytes in and outside the sinuses. No ink particles. Hematoxylin and eosin stain; × 30.

Fig. 4.—Popliteal lymph node, draining the right hindpaw, 15 hours after thermal injury of the paw which, two days earlier, was subjected to a thermal preirritation. Note the architecture of a normal lymph node. Ink particles engulfed by macrophages in the sinuses. Hematoxylin and eosin stain; × 30.

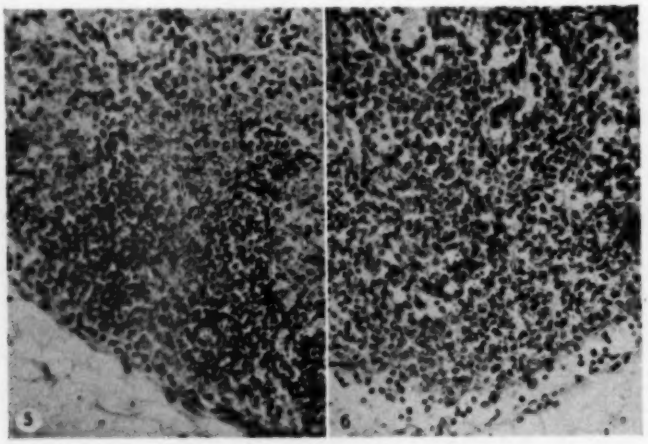

Fig. 5.—Section of Figure 3; × 120.
Fig. 6.—Section of Figure 4; × 120.

architecture of a normal lymph node (Figs. 4 and 6). India ink particles engulfed in phagocytes are seen in the sinuses of the right lymph node (Figs. 4 and 6) but none in the left (Figs. 3 and 5).

COMMENT

The cytologic changes and hemorrhages in the lymph nodes draining the burned areas suggest the action of a toxic substance. It is to be assumed that such a substance was liberated in the tissues under the influence of the thermal irritation and then carried on to the lymph nodes by the lymph flow.

The absence or the marked reduction of pathologic changes in the popliteal lymph nodes after heating of the tributary paws, if these paws had been subjected to a mild thermal preirritation, give rise to the consideration of two major possibilities as follows: (1) that the tissues of the paw and of the draining lymph nodes developed some kind of resistance against the action of the toxic substance normally originating after thermal irritation; (2) that no active substance appeared after the second burning or that it was immediately inactivated.

The first possibility cannot easily be excluded. Tissues can become indifferent toward the action of inflammatory and necrotizing irritants, such as croton oil,‖ after repeated adminstration. Then, however, marked structural changes occur within the tissues, and granulomatous layers [18] form mechanical barriers around the irritant, thus preventing it from attacking adjacent tissue cells. Such barriers were apparently not formed in the lymph nodes in this experiment; the ink particles migrated freely in the protected side, and, also, no evidence of mechanical barriers was found by microscopic examination of the lymph nodes in the protected side. Furthermore, local preirritation of a rabbit ear with croton oil was inffective in reducing the responsiveness of the tissues to a subsequent thermal irritation.[2] Also, preirritation of rat paws by injecting kaolin or India ink failed to afford protection against subsequent heating.[13] This seems to indicate that preirritation with inflammatory substances does not necessarily cause a decrease in the reaction of the tissues to a subsequent heat iritation or to the action of the "burn toxin." Therefore, the possibility that a resistance against the action of the "burn toxin" developed in the tissues of the preirritated paw and its regional lymph nodes does not appear very likely.

On the other hand, there are some experimental observations in support of the second possibility. Protection against fatal shock in mice due to tourniquet of the hindlegs has been reported when a nonfatal tourniquet was applied to the same legs 21 to 38 days in advance.[19] Beloff and Peters [20] observed that a proteolytic enzyme, found in the skin of rats, was decreased after heating, presumably, according to the authors, because of an increased escape of the proteinase from the cells. They advanced the hypothesis that the escape of the proteinase contributes to the formation of the vesicle, and discussed the relation of the proteinase to the "burn toxin" theories. Beloff and Peters reported also [21] that no skin proteinase inhibitor could be recovered from the burned skin of a rat. They considered this observation to give additional weight to their conclusion that the decrease in proteolytic activity of the skin after burning is mainly due to an escape of the enzyme into the circulation. Further, Rawlinson and Kellaway,[22] in perfusion experiments with the isolated liver, observed a rapid increase in the rate of liberation of inorganic phosphatase, alakline phosphatase, esterase, and proteolytic enzymes when the temperature of the perfusion fluid approached 40 to 42 C. This was assumed to be indicative of cell

‖ References 17 and 18.

damage. With regard to this escape of tissue enzymes after burning, Zamecnik, Stephenson, and Cope [12] studied the peptidase activity in the lymph fluid and found an increased activity in the lymph draining burned areas. Further, Glenn, Peterson, and Drinker [11] observed a pink-colored lymph after local burning. They also described an in vitro hemolytic substance in the lymph collected from burned tissues.

Correlated with these reports, the prevention of cytologic changes and of hemorrhages in the regional lymph nodes in this experiment may indicate that a previous thermal irritation of tissues initiates reactions which lead, in a relatively short time, to the exhaustion or blocking of reactions through which toxic substances are normally formed and/or liberated after burning. Thus, the observations reported and commented upon here may offer an explanation for at least one part of the mechanism underlying topical tissue unresponsiveness to inflammatory and nocrotizing injury in thermally preirritated tissues.

SUMMARY

Strong inflammatory reaction following a thermal irritation of a rat paw (49 C. water for two minutes, under compression of the abdominal aorta) is prevented by a mild thermal preirritation of this paw.

Likewise, the hemolymph node formation (cytologic changes and hemorrhages) in the nodes draining the burned areas does not occur or is markedly reduced if the burned tributary tissues are subjected to a mild thermal preirritation.

An exhaustion or blocking of the formation and/or liberation of intermediary inflammatory substances may at least partially account for the acquired topical unresponsiveness of thermally preirritated tissues to a subsequent stronger heat injury leading to inflammation and necrosis in normal tissues.

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EXPERIMENTAL PRODUCTION OF STRUMA FIBROSA

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VERMILLION, S. DAK.

WHILE undertaking a series of experimental studies which demonstrated that struma lymphomatosa could be produced in male rats,[1] preliminary studies also seemed to indicate that a further prolonged administration of thiouracil might result in an increased fibrosis of the thyroid gland.[2] In view of the above experimental observations and the fact that the weight of clinical opinion regarding the controversy over the separate or combined entity of Riedel's and Hashimoto's diseases seemed to now favor the idea that Riedel's struma might be the end-product of Hashimoto's disease,* it was thought of sufficient interest to continue more extensive studies with the use of a drug such as thiouracil in an attempt to produce fibrous hyperplasia in the thyroid gland of a rat. As far as I am aware, the literature contains few or no data concerning the experimental production of an extensive fibroid hyperplasia somewhat similar to that which has been reported as occurring in Riedel's disease.

MATERIAL AND METHODS

Fifty Sprague-Dawley male rats, with an average weight of 61 gm., were divided into two groups. One group of 35 animals received a 0.2% thiouracil † solution as a substitute for the drinking water. The other group was carried along as the normal litter mate control group. All of the animals were maintained at a fairly constant temperature and were given a normal stock diet of Purina dog chow.

The animals were weighed each month, and the entire series in both groups was killed at the end of 24 months by exsanguination under ether anesthesia. Three experimental and two control rats died during the experimental period, and two rats from each group were also killed 6, 14, and 18 months after the initiation of the experiment.

The animals were examined for gross pathological changes, and the following organs and tissues were removed and fixed in formalin, Bouin's, or Zenker's fixative and embedded in paraffin for histological examination: thyroid, pituitary, adrenals, tongue, gingival tissue, teeth, heart, aorta, lung, liver, spleen, pancreas, seminal vesicles, prostate, testes, tibia, and skin. The thyroid glands, the glands with which this report is chiefly concerned, were stained with hematoxylin and eosin for the most part. Some of the serial sections of the thyroid were stained with Mallory's connective tissue stain.

RESULTS

Adenomatous formations were found in the majority of the thyroid glands of the experimental rats, thus presenting additional evidence that diverse types of tumors may be experimentally produced in the male rat.‡ These neoplasms occurred

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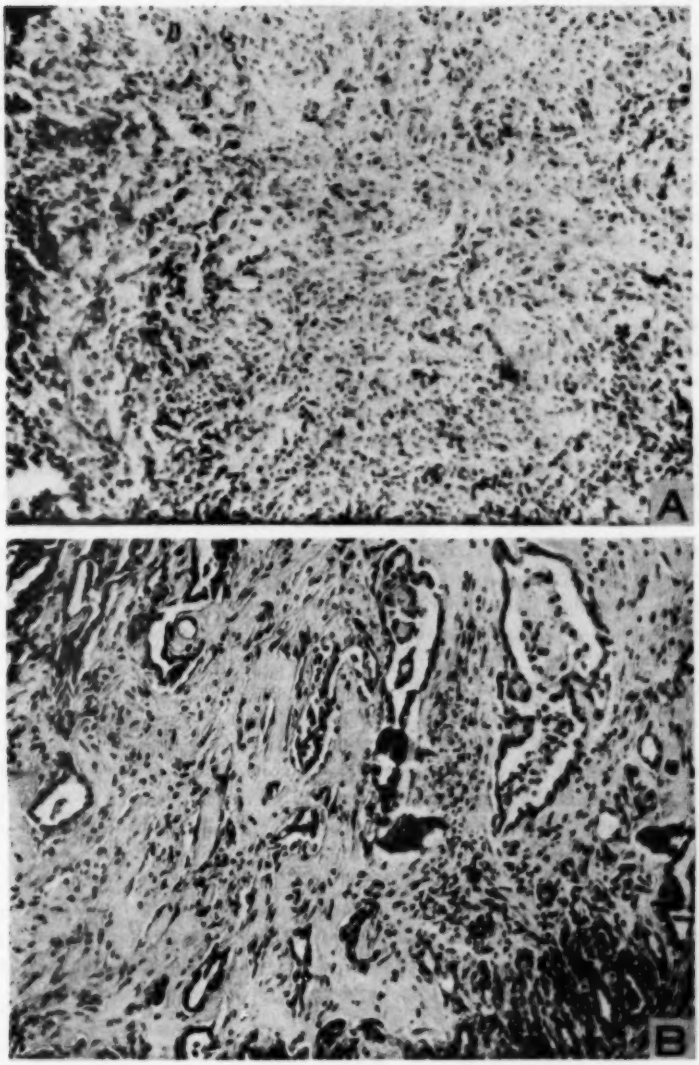
Aided by a research grant from the South Dakota Division of the American Cancer Society.

* References 3-10.

† Thiouracil supplied by Dr. Stanton Hardy, Lederle Laboratories, Pearl River, N. Y.

‡ References 11-15.



A, a fibrolymphoid hyperplasia of the thyroid gland of a male rat following the prolonged administration (18 months) of thiouracil, × 200. *B*, the appearance of fibrous hyperplasia in the thyroid of a rat following thiouracil administration for 24 months, × 200.

in approximately 65% of all the experimental animals in this series. A later report will deal with some of the histological evidence regarding the malignancy of the above formations.

It was apparent that the thyroid glands of six of the rats which had been subjected to thiouracil for 24 months differed considerably from other adenomatous or hyperplastic glands taken from animals which were killed at the same time in this series. A gland taken from an animal which was killed 18 months after the initiation of the experiment was examined and found to be somewhat different, and in a more advanced stage of fibrosis, from those previously reported by me as struma lymphomatosa. In this gland the development of fibrous tissue appeared to be advancing with the gradual departure of lymphoid tissue. It is illustrated, for purposes of comparison, with one that showed a typical fibroid hyperplasia (Fig. A and B).

In six of the rats within the experimental group, the thyroid glands which were removed after 24 months of thiouracil treatment were found to exhibit many characteristics, both macroscopically and microscopically, of those so often reported in the literature as fibrotic, ligneous, or "woody" human thyroid glands. Upon examination of each of the above thyroid glands, prior to fixation for histologic study, it was noted that the glands were enlarged somewhat over the litter mate controls, but they were still approximately 50% smaller than the true adenomatous experimental glands of the same series. The surface of the glands appeared irregularly nodular, with an opaque capsule. A considerable amount of extracapsular adhesion to the immediate surrounding structures was present. In one of the glands it was impossible to remove completely the anterior cervical musculature from the true capsule of the gland. The glands were grayish-white in appearance, with peripheral vascularity somewhat diminished. The glands were very firm in consistency as compared with those glands in which only an epithelial or lymphoid hyperplasia could be demonstrated.

Microscopically, extensive fibrosis of a dense sclerotic type was the most notable feature of these glands. Scattered and relatively few thyroid follicles were to be found. There was very little colloid present in any of the follicles. The epithelial lining cells contained many pyknotic nuclei. In some of the follicles the epithelium assumed a squamous character and often filled the follicular lumen in small sheets. Many follicles were lined with epithelial cells which were hyperchromatic and seemed to be undergoing phases of regression and proliferation. The dense connective tissue was found to have a varying degree of lymphocytic infiltration, and a few plasma cells were in evidence in many regions of the gland. The hyperemic features so characteristic of the early epithelial hyperplasia phase, as well as in the adenomatous thyroids, were absent in the fibroid glands. This latter feature presumably accounted for the difference in size of the true fibroid glands as previously mentioned.

COMMENT

The experimental results presented here suggest that the fibrous hyperplasia, as demonstrated in the thyroid glands of male rats, can be induced through the prolonged administration of an antithyroid drug, such as thiouracil. In view of the fact that previous experimental studies have demonstrated the production of a lymphoid hyperplasia within the thyroid glands of rats receiving thiouracil for 18 months, it is suggested that the additional 6-month treatment with the above

goitrogenic drug resulted in further progressive degenerative changes leading to a fibroid hyperplasia of the thyroids reported here. On a macroscopic and microscopic basis, these glands are similar in many respects to those originally described by Riedel § and one of the four cases described by Hashimoto.[19]

The etiology of struma fibrosa is unknown. Infections of various types have been thought to be the causative factors. If infection were judged as the possible causative factor directly in these experiments, it is rather unlikely that all the litter mate control rats in the present series would have escaped similar infections. If thiouracil were to be considered as an indirect agent responsible for these fibrous tissue changes through infection, it might be explained on the basis of a lowered metabolic activity as produced by the drug and hence a decreased resistance of those animals so involved.

It has been suggested ‖ that overstimulation of the thyroid by the thyrotrophic hormone of the pituitary might be responsible for the thyroid involution and the epithelial degenerative changes as well as the replacement by fibrous tissue. Since antithyroid drugs, such as thiouracil, are known to produce hyperplasia of the thyroid gland, the possibility remains that augmentation of the thyrotrophic hormone ¶ might be directly responsible for fibrous hyperplasia. Consideration must also be taken of the possibility of antithyroid drugs having direct excessive fibrous tissue-stimulating effects as low-grade irritants on a target organ such as the thyroid. Some experimental studies involving this latter aspect of the problem are now under way in this laboratory.

SUMMARY

Some effects of the prolonged administration of thiouracil to male rats were studied. After the drug administration for periods of from 18 to 24 months, 20% of the animals had thyroid glands which exhibited many macroscopic and microscopic characteristics of the late fibrolymphoid stages of struma fibrosa. It is suggested that thiouracil, as administered to male rats, may initiate and maintain a pathological state which, in many instances, may at least simulate those often described as occurring in Riedel's disease.

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‖ References 20-22.
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ATHYROID JOINT DISEASE IN MICE OF VARIOUS AGES

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MICE OF strain DBA radiothyroidectomized after weaning with I^{131} developed a characteristic joint disease distinct from that occurring spontaneously in ageing mice.[1] In order to determine whether or not this athyroid arthropathy was related to disturbances of skeletal growth associated with hypothyroidism, the isotope was administered to adult mice of strain DBA which had ceased to grow. The present report deals with the results of these experiments and with details of the histogenesis of the joint disease.

MATERIAL AND METHODS

One hundred and fifteen male mice of the closely inbred strain DBA raised in our laboratory and kept on a stock diet of Purina laboratory chow and water ad libitum were used. Of these animals, 66 received 200 μc of I^{131} at the age of 1 month (Age Group A), and 49 received 400 μc of the isotope at about 6 months of age (Age Group B).

Some animals of Group A were scheduled to be killed at 6, 8, 12, and 18 months; some of Group B were to be killed at 12, 15, 18, and 21 months. Most of the mice, however, were permitted to live to the end of their natural lives or were killed when they appeared sick, usually because of hypophyseal tumors. At necropsy, the hypophyses were inspected for the presence of tumors. The region of the neck was examined for residual or regenerated thyroid tissue, and whenever such tissue was suspected, it was removed and studied microscopically. Tibiae and femurs including the knee joints were prepared for histologic investigations as described previously.* The sections were stained with hematoxylin and eosin and occasionally with periodic acid—Schiff stain.

OBSERVATIONS

Thyroids.—The thyroids of mice treated with the isotope were usually destroyed. However, here and there, a few small islands of inactive acini could be identified microscopically. The observations agree with those reported by other investigators.†

Hypophyses.—The findings are shown in Table 1. The symbol (+) denotes slight enlargement; + indicates the presence of tumor nodules larger than 2 mm. at least in one diameter.

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This investigation was supported by research grant A-22(C3) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, United States Public Health Service, and by a grant from the American Cancer Society on recommendation of the Committee on Growth, National Research Council.

* Refrences 1 to 3.

† References 4 and 5.

The postirradiation survival of mice of Group B was somewhat shorter than that of mice of Group A. However, this decrease was probably not due to the thyroid deficiency but rather to the fact that the animals were approaching the end of their natural life span.

The total incidence of hypophyseal tumors was 56% in Group A and 10.2% in Group B. Broken down according to comparable postradiation intervals, the incidence was 42.8% at 8.4 months after irradiation in mice of Group A and 4% after a 10.6 months' postradiation interval in mice of Group B; 62.2% of mice of Group A and 36.3% of Group B had pituitary tumors 14 and 13.3 months, respectively, after injection of I^{131}. Thus, 13.3 months after irradiation fewer mice of Group B had pituitary tumors than had mice of Group A after a postradiation interval of 8.4 months. The decreased incidence of I^{131}-induced hypophyseal tumors in mice of the older age group is in keeping with the decrease with age in susceptibility to other neoplasms.[6]

TABLE 1.—*Hypophyseal Findings in Mice Given I^{131} at One and Six Months of Age Respectively*

	Mean Postradiation Interval, Mo.	Experimental Group	I^{131} at, Mo.	No. of Mice	Findings in Hypophyses, %*		
					0	(+)	+
	6.0	A	1	—	—	—	—
	6.0	B	6	13	92.3	7.7	0.0
	8.4	A	1	21	28.6	28.6	42.8
	10.6	B	6	25	64.0	32.0	4.0
	14.0	A	1	45	26.7	11.1	62.2
	13.3	B	6	11	63.7	0.0	36.3
Total	(6-14.0) 12.2	A	1	66	27.3	16.7	56.0
	(6-13.3) 10.0	B	6	49	71.4	18.4	10.2

* (+), slight enlargement; +, tumors at least 2 mm. in one diameter.

Joints.—In contrast to spontaneous osteoarthritic lesions, those due to thyroid deficiency did not begin in the articular surface but rather in the menisci and ligaments. In mice of Group A, the menisci were still in the process of growth and ossification at the time of irradiation. After administration of the isotope, the menisci were enlarged and the ground substance was swollen and contained abundant Schiff-positive material. The enlarged cartilage cells lost their capsules, and the cytoplasm became vacuolated; the nuclei likewise increased in size, and many were finally dissolved. The menisci retained their triangular shape for a considerable length of time, although ossification did not occur or remained incomplete. Under the latter circumstances, the small osseous centers present contained foci of bone marrow as usual. Gradually the process spread to the ligaments. These also were edematous and thickened; the ground substance showed much Schiff-positive material; the cells were swollen and subsequently disintegrated. The degree of involvement varied: In some cases both menisci and both ligaments were affected; in others one ligament or one meniscus was unchanged.

In mice of Age Group B, the menisci were ossified at the time the isotope was administered. Still, the fully developed lesions looked essentially like those in Age Group A: The thin cartilaginous covering of the menisci became enlarged and

merged gradually with the ligaments, which also underwent regressive changes. In addition, the bony tissue lost its typical architecture, and the osteocytes were transformed into cartilage-like and connective tissue cells indistinguishable from those of the ligaments. These cells in turn became hypertrophic and vacuolated. The lesions described so far were classified as "slight."

The "advanced" stage of the disease was indicated by an accentuation of the changes in the menisci and ligaments. Often it was impossible to distinguish between the different types of tissue. The cartilage cells that during the early phase of the disease had been enlarged and vacuolated were liquefied, so that wider areas of tissue broke down and became converted into amorphous eosinophilic material. Many

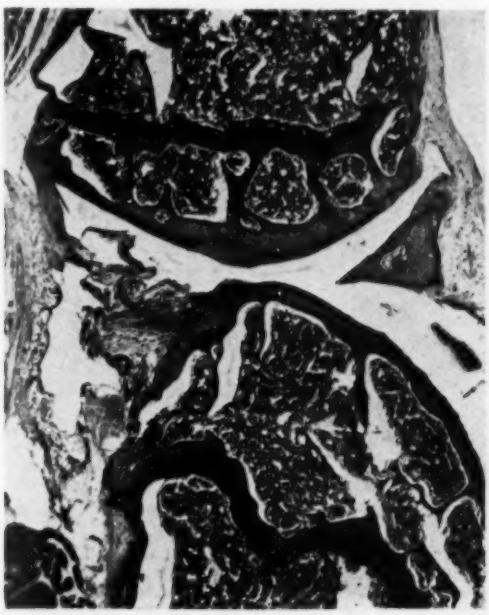

Fig. 1.—Untreated control. Cartilaginous surface is intact. Meniscus at the bottom is ossified and has center of bone marrow. On top, a cruciate ligament is seen. Capsule and synovialis are thin. × 27.

cracks in the sections were additional evidence of these liquefaction processes. The synovialis participated in the process and showed mucoid swelling with the appearance of Schiff-positive material. There was considerable proliferation of fibrous tissue, with thickening of the synovial folds and replacement of the fat pads. This tissue was vascular and occasionally infiltrated with mononuclear leucocytes. The epiphyses, already flattened as a typical effect of thyroid deficiency, were further distorted by fibrous tissue penetrating into the epiphyseal bone marrow. Ultimately, the cartilage of the articular surface became involved and reacted with hyperplasia and hypertrophy. The final result of all these changes was a complex but typical picture characterized by obliteration of the joint cavity and ankylosis. In mice of Group B that lived to a comparatively old age, these athyroid articular changes were associated with osteoarthritic lesions, consisting of proliferation, regressive change,

and ulceration of the cartilage of the surface. Yet even in these "mixed" types of arthropathy, the changes due to athyroidism could usually be distinguished from those of spontaneous degenerative joint disease. Some of the characteristic changes are illustrated in Figures 1, 2, and 3.‡

Incidence of Athyroid Joint Disease.—The incidences of the disease in animals of the two age groups are given in Table 2.

An analysis of the findings in the individual groups shows that a peak was reached about 8 to 10 months after the injection of I^{131}. The incidence had a high near 95 and 64%, respectively, depending on whether the isotope was given at 1 or 6 months of age. With increasing duration of the athyroid state, however, neither

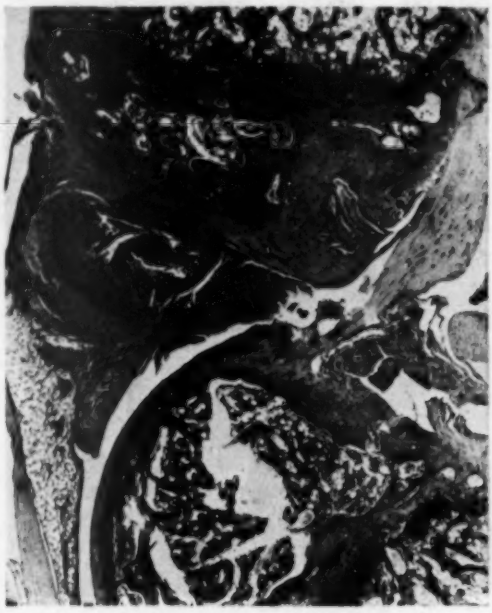

Fig. 2.—Advanced athyroid joint disease after injection of I^{131}: swelling, degeneration, and fibrous transformation of meniscus at top; thickening of ligaments and fibrosis of synovialis which penetrates into flattened epiphysis of tibia; adhesions between meniscus and tibial surface. × 33.

the incidence nor the severity of the joint disease increased much further. The over-all incidence irrespective of age at death was 93.9% for the mice of Group A and 49% for those of Group B. This represents an almost 50% reduction in the susceptibility of animals treated with the isotope in early adult life as compared with that of growing animals. However, the percentage of mice showing advanced articular changes was similar, namely, between 34.8 and 30.6% in mice of both age groups.

For an evaluation of the effects of I^{131}-induced athyroidism on the joints, it is more important to compare groups of animals in which the thyroid deficiency existed

‡ Figures 1, 2, and 3 show sections through knee joints of male mice of strain DBA.

for comparable periods of time than to compare animals of similar mean age at death. This stipulation is most closely approximated by two sets of animals. The first one comprised mice of Group A surviving 8.4 and mice of Group B surviving 10.6 months after irradiation; the second set was composed of mice of Group A with a mean postradiation survival of 14 months and animals of Group B with a mean survival of 13.3 months: 8.4 months after the injection of I^{131}, 90.5% of the mice of Group A had athyroid joint disease, while 10.6 months after injection of the isotope only 64% of mice of Group B were thus affected. This decrease in the incidence of the joint disease by about one-third is even more impressive if one takes into account the fact that the animals of Group B survived irradiation 2.2 months longer than mice of Group A. Similarly, in animals living 14 and 13.3 months after

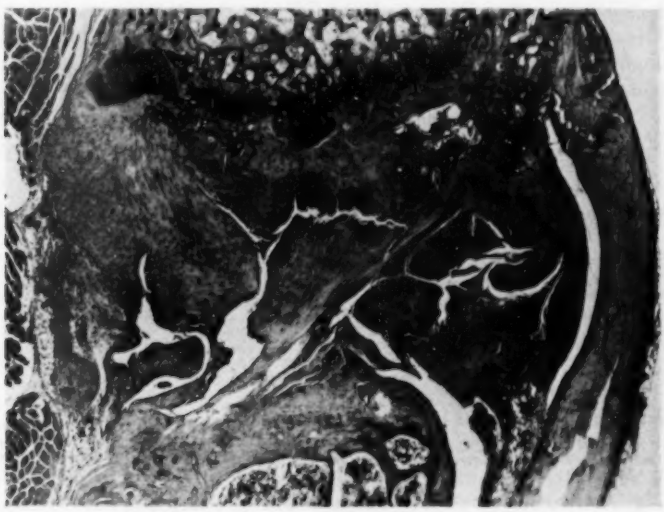

Fig. 3.—Same experiment as in Figure 2. Joint as a whole is markedly enlarged and ankylosed. Meniscus at right is hardly recognizable as such but appears as fibrocartilaginous mass; ligaments and capsule are markedly thickened; synovialis is fibrosed. Epiphysis of tibia (upper half of picture) is distorted to a point where almost entire epiphysis is replaced by fibrous tissue, which has reached cartilaginous epiphyseal growth zone. Note also persistence of epiphyseal cartilage and of thickened metaphyseal spongiosa. × 33.

injections of I^{131}, the incidence of the arthropathy was 95.6% (Group A) and 63.6% (Group B), respectively.

In Table 3, the incidences of osteoarthritic lesions in untreated and radiothyroidectomized male mice of strain DBA are given.

In none of the mice of Group A was athyroid joint disease associated with osteoarthritis, although in untreated controls the incidence of spontaneous osteoarthritis ranged from 10 to 32.5%. By contrast radiothyroidectomized animals of Age Group B developed osteoarthritis in addition to the athyroid articular lesions. The incidence of osteoarthritis increased with advancing age: It rose from 23.1% at a mean age of 12 months to 48% at 16.6 and to 72.7% at 19.3 months of age. The over-all incidence irrespective of age was 47.1%; that is, about twice as high as that seen in control mice of this strain (22.5%).

Tables 4 and 5 were prepared in order to permit an evaluation of the significance of the hypophyseal changes in the development of the athyroid arthropathy. Since hypophyseal tumors arising after radiothyroidectomy secrete large amounts of thyrotrophin, this hormone might in some way take part in producing the changes

TABLE 2.—*Incidence of Athyroid Joint Disease in Mice of Strain DBA Given I^{131} at One and Six Months of Age, Respectively*

	Experimental Group	I^{131} at, Mo.	No. of Mice	Mean Age at Death, Mo.	Mean Survival After I^{131}, Mo.	Mice with Joint Disease		Mice with Advanced Joint Disease	
						No.	% of Total	No.	% of Total
	A	1	21	9.4	8.4	19	90.5	7	33.3
	B	6	13	12.0	6.0	1	7.7	1	7.7
	A	1	45	15.0	14.0	43	95.6	16	35.6
	B	6	25	16.6	10.6	16	64.0	10	40.0
	A	1	—	—	—	—	—	—	—
	B	6	11	19.3	13.3	7	63.6	4	36.4
Total	A	1	66	13.2	12.2	62	93.9	23	34.8
	B	6	49	16.9	10.9	24	49.0	15	30.6

TABLE 3.—*Incidence of Degenerative Joint Disease in Untreated Male Mice of Strain DBA and Those Radiothyroidectomized at Six Months of Age*

Age Group, Mo.	Untreated Mice *		Radiothyroidectomized Mice	
	Mean Age, Mo.	Incidence, %	Mean Age, Mo.	Incidence, %
7-12	9.3	10.0	12.0	23.1
13-18	17.6	32.5	16.6	48.0
19-21	19.7	30.0	19.3	72.7
Total 7-21	14.5	22.5	16.9	47.1

* These findings have been reported more fully elsewhere (Silberberg, R., and Silberberg, M.: Growth **14**: 213, 1950).

TABLE 4.—*Incidence of Hypophyseal Findings in Relation to Presence of Advanced Athyroid Joint Disease and of Unchanged Joints, Respectively*

Age Group	I^{131} at, Mo.	Mice Showing Advanced Joint Disease				Mice Free of Joint Disease			
		No. of Mice	Findings in Hypophyses *			No. of Mice	Findings in Hypophyses *		
			0	(+)	+		0	(+)	+
A	1	23	3	2	18	4	1	3	0
B	6	15	8	5	2	25	24	1	0

* (+), slight enlargement of hypophysis; +, hypophyseal tumors at least 2 mm. in one diameter.

observed. The symbol (+) denotes slight and + designates advanced athyroid joint disease. Table 4 shows the incidence of hypophyseal changes in relation to the presence of advanced articular lesions and of unchanged joints, respectively.

In mice of Group A, there was a strong positive correlation between the occurrence of hypophyseal tumors and advanced athyroid arthropathy: 18 of 23 mice with articular lesions also had hypophyseal tumors, while of animals free of joint

disease none had developed hypophyseal growths. In mice of Group B, the role of the hypophysis in relation to the joint disease was less obvious than in Group A: Only 2 of 15 mice of Group B with advanced arthropathy also had hypophyseal tumors. Still, mice free of joint disease likewise had no hypophyseal neoplasms.

In Table 5, the incidence of joint disease in relation to the presence of hypophyseal tumors or grossly unchanged hypophyses are given.

About one-half (18 of 37) of the mice of Group A with hypophyseal tumors also had advanced athyroid arthropathy, while only 2 of 18 mice with unchanged hypophyses showed advanced articular lesions. In Group B, again, about one-half (3 of 5) of the mice with hypophyseal tumors had advanced joint disease, while in the absence of hypophyseal changes 8 of 34 mice had advanced articular lesions.

COMMENT

Male mice of strain DBA radiothyroidectomized with I^{131} developed a characteristic arthropathy. It differed from the joint disease occurring spontaneously in aged mice in that it involved primarily the menisci, ligaments, and synovialis and

TABLE 5.—*Findings in Joints of Mice with Hypophyseal Tumors and Grossly Unchanged Hypophyses*

		Mice with Hypophyseal Tumors				Mice Without Hypophyseal Changes			
			Findings in Joints *				Findings in Joints *		
Age Group	I^{131} at, Mo.	No. of Mice	0	(+)	+	No. of Mice	0	(+)	+
A	1	37	0	19	18	18	1	15	2
B	6	5	0	2	3	34	23	3	8

* (+), athyroid joint disease slight; +, athyroid joint disease advanced.

extended only secondarily to the articular cartilage; in addition the increased amount of Schiff-positive material in the articular tissues was striking. The formation of such substance may have been due to factors similar to those that bring about the appearance of such material in the cardiac muscle of myxedematous individuals.[7]

The joint disease was more frequent in mice thyroidectomized early in life than in mice receiving the isotope after cessation of linear growth. There is thus some similarity of the effects of thyroid deficiency on the joints of animals and of man.§ Thyroid deficiency has for some time been recognized as a factor in juvenile arthropathies, although there has been reluctance to attribute joint disease of the adult to lack of thyroid hormone. Whether or not the analogy between the findings in animals and in man can be carried further remains to be seen. The present observations pose several questions: 1. Does the hyperpituitarism induced by I^{131} play any role in the evolution of the athyroid arthropathy? 2. Why do the joints of mice injected with I^{131} after cessation of growth react less promptly to the athyroid state than those injected after weaning? 3. Is there any causal relationship between the athyroid joint disease and the osteoarthritic lesions occurring spontaneously in aged mice?

The decline in incidence of the athyroid arthropathy with advancing age at the time of irradiation parallels the decline of hypophyseal tumors seen under the same conditions. A high incidence of joint disease coincided with a high incidence of

§ References 8 to 11.

hypophyseal neoplasms, and mice without articular lesions usually showed few hypophyseal changes. However, the reverse was not true: Not all mice with hypophyseal tumors had advanced articular disease, and about one-fourth of all mice with grossly unchanged hypophyses had severely diseased joints. Therefore, the arthropathy does not seem to have been dependent on hormones produced by the hypophyseal tumors, and the simultaneous occurrence of high or low incidences, respectively, of hypophyseal tumors and of arthropathy in some groups of animals may have been largely coincidental. Whether or not thyrotrophic hormone may contribute to the development of articular lesions will have to be decided by further experiments. That such a role of thyrotrophin would be limited is suggested by the results of similar experiments in mice of strain C57BL.[3] In these, athyroid joint lesions were absent or very slight in spite of the fact that in this strain hypophyseal tumors were almost twice as frequent as in mice of strain DBA.

The decreased susceptibility of adult mice to the arthropathy may be related to changes in the connective tissue, which is more abundant and more rigid in old than in young tissues. Since thyroid hormone promotes condensation of the ground substance, lack of this secretion should be conducive to swelling and vacuolation, as seen in cartilage and ligaments of mice treated with I^{131}. In older mice the comparatively inert connective tissue, with firmer cohesion of matrix and cells, may oppose a stronger resistance to the changes caused by the hormonal deficiency than the tissues of younger animals would.

In mice of strain DBA radiothyroidectomized early in life osteoarthritis did not develop, even though the animals lived to an age at which untreated controls show such lesions. Radiothyroidectomy carried out at an early age thus decreased the incidence of osteoarthritis, as it did in mice of strain C57BL.[3] This effect is considered to be related to the retardation of skeletal growth and development associated with thyroid deficiency. The absence of osteoarthritis in joints severely damaged by thyroid deficiency indicates that factors other than purely mechanical ones have to come into play before degenerative joint disease develops.

In aged mice of strain DBA radiothyroidectomized after cessation of growth, the incidence of osteoarthritis was increased over that of the spontaneous disease. This finding supports the view that on the one hand a fully developed cartilage is a prerequisite for the development of osteoarthritis [12] and that on the other the course of degenerative joint disease may be modified by thyroid deficiency.

SUMMARY

In male mice of strain DBA, radiothyroidectomy performed after weaning called forth a typical arthropathy. Radiothyroidectomy carried out in young adult mice was less effective in producing this lesion than it was in growing animals. Mice radiothyroidectomized during the growth period did not develop degenerative joint disease in old age as do aged mice with intact thyroids. By contrast, radiothyroidectomy performed in young adult male mice of strain DBA increased the incidence of degenerative joint disease over that seen in untreated controls. The decline in the incidence of athyroid joint disease with increasing age at the time of irradiation parallels the decrease of hypophyseal tumors occurring under the same conditions. It is, however, unlikely that a causal relationship exists between the hypophyseal neoplasms and the athyroid joint disease.

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BETA GRANULES OF ISLETS OF LANGERHANS OF RAT

A Study of Their Development Under Normal and Abnormal Conditions

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A RELIABLE stain is now available for the demonstration of beta granules in the islets of Langerhans.* Since these granules presumably represent stored insulin,† a method is at hand to study the storage of insulin in the fetus and the factors that affect the production of insulin in the fetus.

This paper deals with the normal development of beta granules in fetal and newborn rats' pancreases and the experimental conditions which affect their development.

EXPERIMENTS

NORMAL DEVELOPMENT OF THE BETA GRANULES IN THE RAT.—A large group of female albino rats were isolated from male rats for a period of three weeks. One hundred nonpregnant females weighing between 150 and 250 gm. were then bred to males during a period of six months. The method used was to isolate five females with two males for 24 hours. The males were then removed, and the pregnancy was considered as beginning 12 hours after the addition of the male in the females subsequently proving to be pregnant. The pregnancy was terminated by removing the fetuses by Caesarean section. The period studied was from the 7th to the 21st day of gestation. Newborn rats up to 3 weeks of age were also studied. All the pancreases were prepared in a similar manner by fixing in 10% formalin and staining with our modification [3] of Gomori's chrome alum hematoxylin stain ‡ for the demonstration of the alpha and beta granules in the islets of Langerhans.

This study is concerned with development of the beta granules and not with the development of the beta cell. In addition, the development of the alpha cell granules was studied.

The first beta granules were found to appear on the 20th day of gestation. Therefore, a description of the islets from the 19th day of gestation onward will be given.

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This work was done under the guidance and with the advice of Dr. E. T. Bell, professor emeritus of the Department of Pathology. It was supported in part by a research grant (A-574) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, United States Public Health Service.

* References 1, 2, and 3.

† References 4 to 7.

‡ References 1 and 2.

Nineteenth Gestational Day.—The islet tissue had a very loose myxoid appearance. The cells had a good deal of cytoplasm about the nuclei. Many of the cells were spindle-shaped. The islet tissue was very prominent and ramified throughout the pancreas and anastomosed with other projections of islet tissue. No granules of either the alpha or the beta type could be recognized.

Twentieth Gestational Day.—The islet tissue was much more compact than that seen in the 19th day specimen. There was much less ramification of the islet tissue in that the islets were surrounded by acinar tissue creating real "islets." Most of the islet tissue contained large hydropic cells. Here and there in an islet could be seen a beta cell with the characteristic blue-staining granules. The vacuolated appearance of the cells destined to become filled with beta granules was strikingly similar to the hydropic cells described by me with the use of exogenous insulin and starvation.[8] Alpha granules were not seen in any of the cells. It should be emphasized that the cells which were interpreted as alpha cells, namely, those along the periphery of the islet, also showed a severely vacuolated (hydropic) cytoplasm. This appearance was in contrast to the picture seen in rats treated with insulin or starved, who showed normally granulated alpha cells.

Twenty-First Gestational Day.—There was a distinct increase in the amount of beta granulation. Most of the islets now contained a substantial number of beta granules. The cells of some islets, however, were not fully granulated. An occasional islet was seen with no granules. The cells not containing any granules or only a few beta granules showed a vacuolated cytoplasm similar to that already described. The cells in the center of the islets granulated before those on the periphery of the islet. The cells destined to become alpha cells still showed no granules in their cytoplasm.

Newborn Rats.—The beta cells were fairly well filled with beta granules. Hydropic cells were still seen, but they had decreased in number. No alpha granules were present; these cells still showed hydropic changes.

Twenty-Four Hours Post Partum.—The beta cells appeared fully granulated, and the hydropic changes had disappeared. The alpha cells did not have granules; these cells had a vacuolated cytoplasm.

Subsequent Course.—Twenty-four hours after birth and subsequently thereafter the beta cells appeared to have their full complement of granules. No other changes were seen after this time. The alpha cells appeared vacuolated until the third day post partum. No alpha granules could be detected until the fourth day post partum. At that time the alpha cells seemed well granulated, and they remained so thereafter.

Effect of Insulin (Given to Mothers) on Fetal Pancreas.—Fifty female albino rats weighing between 150 and 250 gm. were divided into two equal groups designated A and B.

Group A—25 Rats.—These rats were placed in cages with males and allowed to breed. The females received the maximum tolerated dose of protamine zinc insulin (4 units per 75 gm. of body weight) daily. Administration of insulin was continued during the period of pregnancy. Since pregnancy was initiated on different dates in many of the rats, the period of insulin treatment varied from 21 to 60 days. If the gestational period is taken as 21 days, then the rat receiving insulin for 60 days was given insulin for 39 days before pregnancy began. Many of the pregnant rats died

during the experiment from hypoglycemia. There were 50 newborn rats. Two rats were killed immediately after birth; thereafter, two additional rats were killed at 24 hour intervals, so that a series of pancreases was obtained beginning immediately at birth and extending to 24 days post partum. The pancreases were stained as already described to demonstrate the beta granules.

Group B—25 Rats.—These female rats were bred to males as described for Group A. Each rat received 3 cc. (120 units) of regular insulin each day. Administration of insulin was continued during the period of pregnancy. None of this group died of hypoglycemia during the experiment. It should be pointed out that adult rats are able to tolerate very large amounts of regular insulin,[9] in contrast to their reaction to protamine zinc insulin.

Results.—The beta granules appeared to develop normally in both Group A and Group B. No differences in development of the beta granules could be detected when results were compared with those in the normal control group.

Effect of Starvation and Glucose on the Development of the Beta Granules.—A single large litter of newborn rats (10 animals) was separated from its mother and divided into two equal groups designated C and D.

Group C.—Of five newborn albino rats, one was killed immediately after birth. The rest were starved. Three rats were killed at 18, 24, and 36 hours, respectively, after birth. The fifth rat was found dead at the end of 36 hours. The pancreases were stained as already described.

Group D.—Five newborn albino rats were treated in a manner identical to that described for Group C except that 0.5 cc. of 5% glucose was given subcutaneously three times a day.

Results.—The beta granules of Groups C and D developed normally. No difference between these two groups and the normal group could be detected.

Effect of Maternal Diabetes on the Islets of Langerhans of the Fetus.—Fifty female rats were made diabetic with alloxan. They were then bred with males. Over a period of six months only three of the females carried litters to term. A few of the female rats died during pregnancy. Most of the females did not become pregnant, probably because of the diabetes. The offspring of these three litters were nursed by the diabetic mothers; they were killed at intervals so that pancreases from the newborn period to two weeks post partum were obtained. The development of the beta granules in this group was compared with that in the normal control group.

Results.—The pancreas from the rat killed immediately after birth showed a total lack of granulation of the beta cells. Interestingly enough, the alpha cells were very prominent. Some islets were made up completely of alpha cells. This prominence of the alpha cells was in contrast to the appearance of the alpha cells in the normal newborn rat, which were first recognized on the fourth day after birth. The beta cells showed a rather marked hydropic appearance. The beta granules first appeared in the rat killed 24 hours after birth, while the rats killed 48 or more hours after birth showed complete granulation of the beta cells. The pancreases from the rats killed two and three days after birth did not show granulated alpha cells. Those killed after the fourth postpartum day showed granulated alpha cells. No abnormalities in the islets could be seen aside from the delay in appearance of the beta granules and the early appearance of granulated alpha cells in a single rat.

Effect of Cortisone on the Development of the Beta Granules.—Twenty-five newborn rats were injected with cortisone immediately after birth and daily thereafter. They were nursed by their own mothers. Each day 0.1 ml. (2.5 mg.) of cortisone was given until the rat was killed or found dead. Many of the rats were found dead with multiple abscesses of the subcutaneous tissue and visceral organs after the fifth day of treatment. From the fifth day on the treated animals showed progressive emaciation and dehydration. The longest survival period was nine days. The series consisted of rats killed or dead from 24 hours to 9 days following birth. The pancreases were stained as already described. Only one rat of the entire series showed a partial degranulation. It should be pointed out that the beta granules stained well as long as the nuclei of the beta cells were recognizable. This animal was found dead on the eighth day following cortisone treatment. The rest showed no variation from the normal series previously described.

COMMENT

The observations of myself and my co-workers on the development of the beta granules in the rat's pancreases with use of our staining technique are in agreement with Hard's [10] findings with the neutral gentian stain of Bensley. The development of the beta granules in the rat pancreas late in the third trimester of pregnancy contrasts sharply with their early appearance in the human pancreas, which we have found to occur in the first trimester. This fact suggests that insulin secretion appears relatively much earlier in humans as compared with rats.

The hydropic appearance of the developing beta cells of the fetal rat is interesting, since this change is usually regarded as a degenerative change.

The lack of effect of exogenous insulin on the development of the beta granules of the fetus probably means that little or no insulin crosses the placental barrier.

It is surprising that starvation of the newborn rat does not prevent the development of beta granules, since it has been shown that starvation will degranulate an already fully granulated beta cell in the adult pancreas. § This observation indicates clearly that the development of beta granules in the rat pancreas is not due to the stimulating effect of the mother's milk.

Our results with the use of cortisone were unsatisfactory. Cortisone causes emaciation and the development of multiple abscesses. We were unable to produce hydropic changes of the beta cells as observed in rabbits by Warren.[12]

SUMMARY

1. The beta granules of the islets of Langerhans first appear in the fetal rat pancreas on the 20th day of gestation. The beta cells become fully granulated 24 hours after birth, 48 hours after their initial appearance. The beta granules appear relatively much later in the rat (late third trimester) than in the human (first trimester).

2. The alpha cells become granulated 96 hours after birth.

3. Insulin given to pregnant rats does not affect the developing islets of Langerhans of the fetus.

§ References 8 and 11.

4. Neither starvation nor injected glucose seems to affect the developing beta granules of the newborn rat.

5. Maternal rat diabetes mildly inhibits the development of the fetal beta granules.

6. Cortisone does not appear to exert a significant effect on the developing beta granules of the newborn rat.

Miss Lois Folie furnished the necessary technical assistance.

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EXPERIMENTAL BACTERIAL ENDOCARDITIS IN ALTITUDE RATS

Development and Regression of Cardiac Lesions, Including Lesions in Rats Treated with Penicillin

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IN PREVIOUS reports * it was shown that bacterial endocarditis may be readily induced in rats by intravenous injection of broth cultures of viridans streptococci if the animals (altitude rats) have been acclimatized previously to a simulated high altitude. In this report we shall describe the development of the cardiac lesions in such altitude rats and the regressive changes, particularly those following treatment with penicillin. Certain findings in this study may elucidate some of the lesions in human endocarditis and causes of treatment failures.

MATERIALS AND METHODS

Several hundred animals from selected experiments were used to study the development of the valvular lesions. In one typical experiment, 183 young male rats of the Holtzmann strain received 30 daily four-hour exposures in a low pressure chamber to a simulated altitude of 25,000 ft. They then were given a single intravenous injection of 0.5 cc. of a six-hour broth culture of Streptococcus faecalis (Lancefield D).† Treated animals were given 200 mg. per kilogram of sodium penicillin G four times daily and 20 mg. per kilogram of probenecid (Benemid) ‡ twice daily for 1 to 11 days beginning 12 and 108 hours after inoculation. Untreated animals and animals treated with penicillin, usually three to six per group, were killed for histologic and bacteriologic studies at intervals ranging from 12 hours to 35 days after inoculation. As reported previously,[3] these studies revealed that nearly all rats developed a bacterial endocarditis. In two other selected studies, similarly injected altitude rats were not treated, and in two others, treatment was begun 20 and 72 hours after inoculation.

Microscopic studies were made of the hearts of several hundred additional animals from other experiments.§ These rats died or were killed at varying intervals after multiple inoculations of Str. faecalis, Streptococcus mitis, and other strains of viridans streptococci. These rats were used to study more fully some of the more severe and less common cardiac lesions. Rats not inoculated with bacteria were examined after 30 exposures to 25,000 ft. They did not show sterile vegetations or other severe valvular lesions such as those described in rats exposed for three to six months.[7] Other controls were maintained at ground level and killed at intervals after single and multiple bacterial inoculations.

This is a portion of a paper presented at the Fiftieth Annual Meeting of the American Association of Pathologists and Bacteriologists, St. Louis, April 3, 1953.

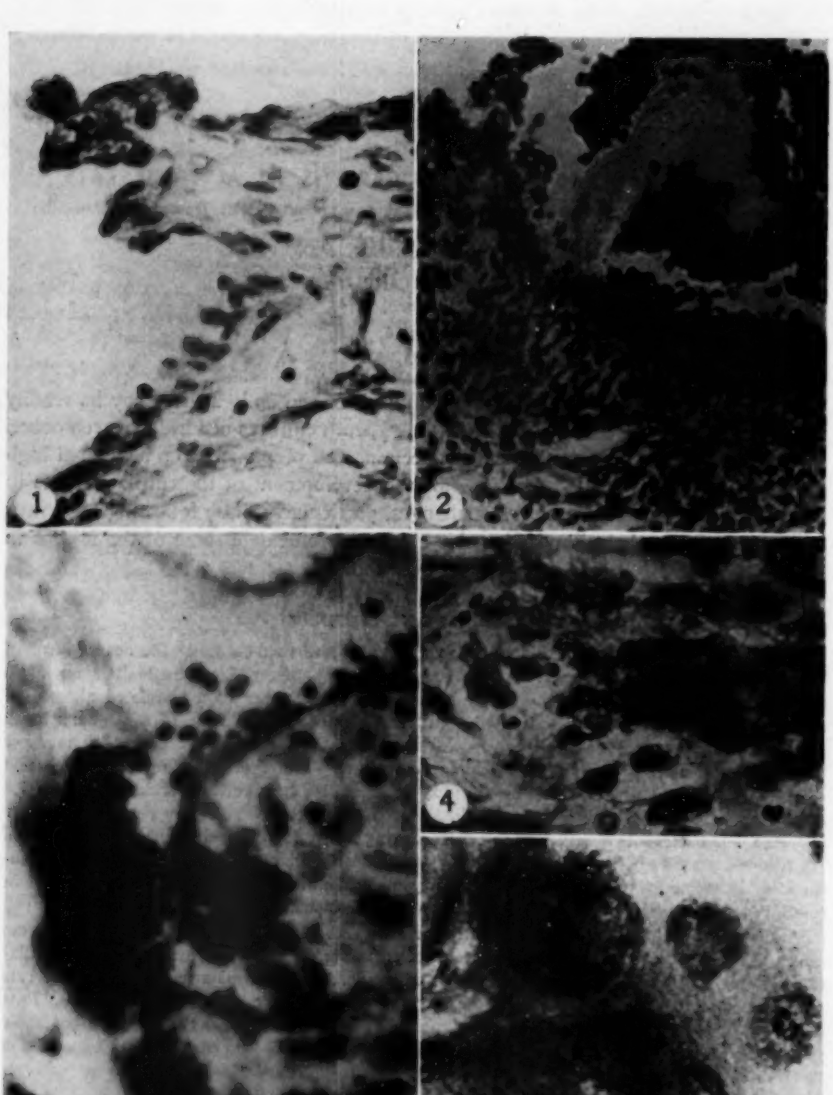
From The National Institute of Arthritis and Metabolic Diseases and the National Microbiological Institute, National Institutes of Health, United States Public Health Service.

* References 1 to 6.

† Type determined by Dr. R. N. Cole, National Microbiological Institute.

‡ Furnished by Sharp & Dohme, Inc.

§ References 1 and 2.



Figures 1 to 5

(See legends on opposite page)

The methods used in exposing the animals ‖ and in preparing and inoculating the bacterial cultures [1] have been described previously. After fixation in 10% buffered formalin (pH 7), the hearts were hemisectioned in a plane passing through the four cardiac chambers. Standard procedures being used, each half of the heart was embedded in paraffin, and skip serial sections were prepared by cutting ribbons of four to six sections each from several different levels.

The sections were stained routinely with hematoxylin azure eosinate and in selected instances with a variety of stains to demonstrate bacteria, collagen, fibrin, elastic and reticulum fibers, and hemosiderin.[9] The method of Rinehart and Abul-Haj [10] was used to demonstrate material staining like acid mucopolysaccharides.

PATHOLOGIC OBSERVATIONS

The rate of development and the severity of the valvular lesions varied considerably under different experimental conditions and in individual animals, but the pathologic features were essentially similar at a given stage of development. The infected animals developed a valvulitis usually associated with bacterial vegetations. The infection involved chiefly the mitral and aortic valves; frequently both; occasionally the tricuspid and pulmonary valves, and, usually by contiguity, the mural endocardium and wall of the aorta. The severity of the lesions, if multiple, often varied considerably in different leaflets and in different portions of the same leaflet. Severe lesions tended to occur most frequently on the auricular surface of the mitral valve and both surfaces of the aortic valve.

Progressive Valvular Changes in Untreated Altitude Rats Following Single Inoculation of Str. Faecalis

First 24 Hours.—The earliest change, noted one to six hours after inoculation, consisted of an abnormal number of white blood cells in the cardiac chambers focally marginating the endothelium, particularly of the leaflets. At 8 to 12 hours, there were one or more small discrete lesions occurring anywhere along the surface of one or more leaflets. Scattered lymphoid cells and neutrophiles were found in and along the surface of these lesions. The endothelium was usually swollen and sometimes partially exfoliated. A small amount of deeply eosinophilic fibrillar material

‖ References 7 and 8.

Explanation of Figures 1 to 5

Fig. 1 (Rat 551).—Auricular surface of mitral leaflet of altitude rat killed 12 hours after single inoculation of Str. faecalis. Note scattered lymphoid cells and neutrophiles and, in upper portion of figure, some fibrin-like material along surface and beneath endothelium. Hematoxylin azure eosinate; × 500.

Fig. 2 (Rat 451).—Tricuspid leaflet 20 hours after inoculation of Str. faecalis, showing on auricular surface small vegetation, marginated by erythrocytes, containing dark triangular colony of bacteria in midlower portion. Underlying leaflet is densely infiltrated by neutrophiles and large mononuclear cells. Hematoxylin azure eosinate; × 545.

Fig. 3 (Rat 563).—Fold of aortic leaflet 60 hours after inoculation of Str. faecalis. Large dark mass on left is colony of bacteria lying in lower portion of a vegetation which covers sharply delineated upper surface of leaflet. Dark round masses to right of colony are bacteria-laden macrophages in valvular stroma. Below colony are partially detached bacteria-laden lining endothelial cells. Hematoxylin azure eosinate; × 545.

Fig. 4 (Rat 577).—Fold of mitral leaflet in rat dying 84 hours after inoculation of Str. faecalis, showing valvular stroma changes at base of bacterial vegetation. Hematoxylin azure eosinate; × 535.

Fig. 5 (Rat 577).—Same rat as in Figure 4, showing macrophages in bacterial colonies of mitral vegetation. Hematoxylin azure eosinate; × 535.

was seen along the surface and occasionally beneath apparently intact endothelium (Fig. 1). Bacteria were found only occasionally in endothelial cells. Changes in the valvular stroma were similar to but less marked than those found at 20 hours.

In animals killed 20 hours after inoculation, the inflammatory lesions in the leaflet were larger, often confluent, and densely infiltrated by neutrophiles and fewer large mononuclear cells and lymphoid cells. Some lesions were surmounted by a vegetation (Fig. 2). This consisted of a small irregular mass of eosinophilic material containing a small colony or a few chains of streptococci, and neutrophiles and large mononuclear cells; a few cells contained bacteria. The affected leaflet was irregularly thickened owing to edema and other changes, described hereafter, occurring in the valvular stroma, particularly in and near the lesions. Most constantly, the stroma cells were greatly increased in number and size and widely separated by an increased amount of abnormally clear intercellular matrix. Mitotic figures and cells with two or more nuclei were seen occasionally. Some of the stroma cells assumed stellate or rounded forms grading into macrophages. Others showed condensation of the chromatin into a straight or undulating bar similar to that seen in Anitschkow myocytes, while a few cells were necrotic. Occasionally, the cells at the base of vegetations were arranged in palisade formation with their long axes at right angles to the surface. The inflamed leaflet often revealed an increase of metachromatic material and material staining for acid mucopolysaccharides. Some leaflets showed irregular clear spaces (Fig. 1) or small deposits of edema fluid and occasionally of eosinophilic granular material. The elastic fibers coursing through or beneath the lesions were often widely separated and focally disorganized.

After 24 Hours.—During the next few days, the inflammatory reaction and the changes in the stroma became more intense and widespread, often involving nearly the entire leaflet and extending into the valvular rings, the interstitial tissue of the adjoining myocardium, the epicardium, and the adventitia and root of the aorta. The leucocytic infiltration was particularly intense at the base of vegetations, where abscesses occurred at times. Elsewhere, the leucocytes were irregularly disposed singly and in small clumps between the stroma cells. Swelling and separation of the stroma cells and alterations in their shape were most marked at the base of vegetations; in other areas, the cells were greatly increased in number but compactly disposed. Masses of bacteria were occasionally found in intact or detached lining endothelial cells and in superficial stroma cells and large mononuclear cells (Fig. 3). Vegetations increased in number and size as the interval after the inoculation increased and were found in nearly all animals that died or were killed 96 hours after inoculation.

Death from infection usually occurred 4 to 10 days after inoculation. Animals with such severe infections frequently had huge vegetations involving both surfaces of several leaflets and filling much of the left auricle or ascending aorta (Fig. 6). Some vegetations extended by contiguity to the adjoining mural endocardium, the chordae tendineae, the intima of the aorta, or the surface of an adjoining leaflet. The changes in the leaflet were similar to but usually more severe than those seen earlier. In a few instances, extravasated red blood cells were seen beneath the endothelium of the auricular surface of the mitral or tricuspid valve.

Structure of Vegetations.—The vegetations were composed of an eosinophilic matrix admixed with bacteria, cells, and debris. Young vegetations (20 to 36 hours

after inoculation) were small and contained relatively few cells and bacteria. Their outer surface was usually smooth, and their base was sharply delineated (Figs. 2 and 3). Older vegetations were larger and often had a highly irregular, deeply fissured outer surface and numerous internal clefts. They usually had a broad, occasionally bifurcated, base, which was poorly delineated from the inflamed leaflet owing to an ingrowth of fibrous tissue.

The matrix of young vegetations (Fig. 2) was fairly homogeneous and closely resembled fibrin morphologically and tinctorially, though the matrix of some stained less intensely with the periodic acid-Schiff and other procedures than the postmortem fibrin clots seen in the cardiac chambers. The matrix of older vegetations was

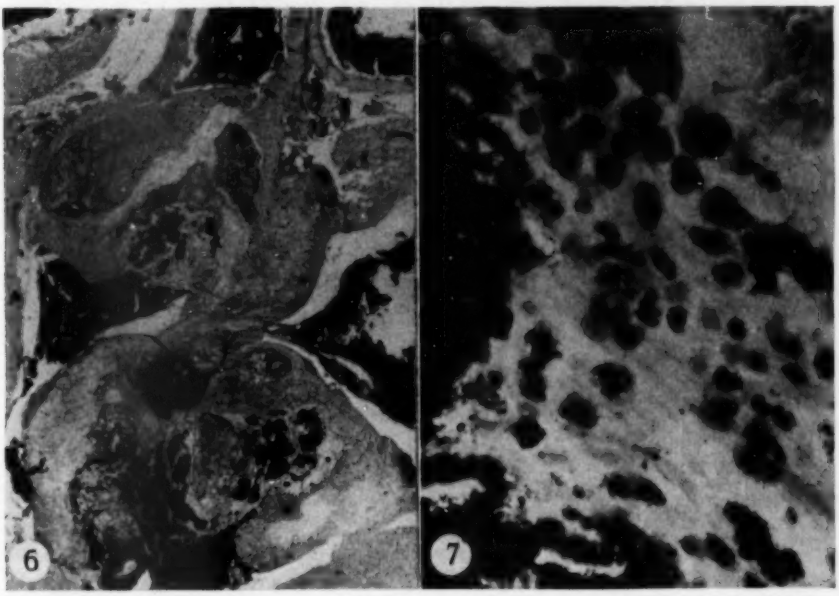


Fig. 6 (Rat 1183).—Rat dying six days after inoculation of Str. faecalis, showing huge vegetations involving aortic and mitral leaflets. Brown-Brenn stain; × 14.

Fig. 7 (Rat 537).—Brown-Brenn stain of mitral vegetation in rat killed seven days after inoculation of Str. faecalis. Note bacteria-laden macrophages grading into satellite colonies; × 500.

less homogeneous and showed many loosely fibrillar, spongy, or vacuolated pale cellular areas. These areas graded into and were separated by deeply eosinophilic compact bands and areas in which were embedded most of the bacteria. The matrix, particularly in the cellular areas, stained faintly or not at all like fibrin. As will be indicated subsequently, it is significant that the matrix of both young and older vegetations did not stain for acid mucopolysaccharides by the method of Rinehart and Abul-Haj even when the subjacent leaflet contained an abundance of such material.

The bacteria were compactly disposed in scattered discrete and confluent colonies, which increased in number and size with the age of infection. They were Gram-

positive, deeply basophilic, and intensely stained by the periodic acid-Schiff and the Rinehart and Abul-Haj procedures, but portions of some colonies stained poorly. The colonies were usually sharply delineated and situated in dense matrix at various levels in the interior of the vegetation and occasionally along its outer surface. Many were surrounded by a cell-free zone. The outlines of macrophages or their nuclei were noted in the interior of some colonies, particularly in the poorly staining portions (Fig. 5). Small satellite colonies graded into bacteria-laden macrophages (Figs. 5 and 7). There were occasional small groups of extracellular streptococci.

Erythrocytes, often partially hemolyzed, were irregularly distributed singly, in small groups, or in larger masses. They were particularly abundant in vegetations with a highly irregular surface and were relatively uncommon in small vegetations.

The cellularity of the vegetations varied markedly but tended to increase with age of infection. Leucocytes and large mononuclear cells were most abundant at the base of vegetations and in the vicinity of the bacteria, but some were scattered irregularly throughout the vegetation. The leucocytes often formed rings around the colonies, and those nearest the colonies were frequently fragmented. Relatively few leucocytes contained bacteria. The mononuclear cells showed various gradations in appearance from lymphoid cells to macrophages and large fusiform cells. As noted previously, some mononuclear cells lay within bacterial colonies.

The vegetations often contained cellular debris and basophilic smudges suggesting lysed colonies or cells and occasionally a few argyrophilic and elastic fibrils. The fibrils were usually found at the base in association with invading fibroblasts, but were occasionally seen in adjoining acellular areas and in detached fragments of valvular stroma.

Regression of Valvular Lesions Following Single Bacterial Inoculation

Untreated Rats.—While the majority of untreated rats showed severe progressive valvular lesions, regressive changes were noted in some killed after the fourth day and occasionally even in animals dying from the infection. Such changes were essentially similar to but generally slower in development than those to be described in rats recovering after delayed treatment with penicillin.

Rats Receiving Early Penicillin Treatment.—When penicillin therapy was instituted 12 or 20 hours after a single inoculation of Str. faecalis, nearly all animals killed 24 and 48 hours after the beginning of therapy displayed evidence of a receding valvulitis. The affected leaflet presented, usually along its free-flowing surface, one or more small areas showing edema, an increase in number and size of the stroma cells, or a small collection of lymphocytes, large mononuclear cells, or neutrophiles. The majority showed no significant pathologic findings after 48 hours, but a few showed sterile vegetations in various stages of organization as late as six days after institution of therapy.

Rats Receiving Delayed Penicillin Treatment.—When penicillin therapy was instituted 72 or 108 hours after the inoculation, marked regressive changes were noted, frequently within 24 hours, and nearly complete regression within a week. Regression was evidenced by destruction of the bacteria, organization of the vegetations, and subsidence of the inflammatory reaction in the valves (Figs. 8, 9, 10, and 11).

Destruction of Bacteria.—The bacteria often disappeared within 48 hours after the commencement of penicillin therapy (Fig. 8). Destruction was accomplished

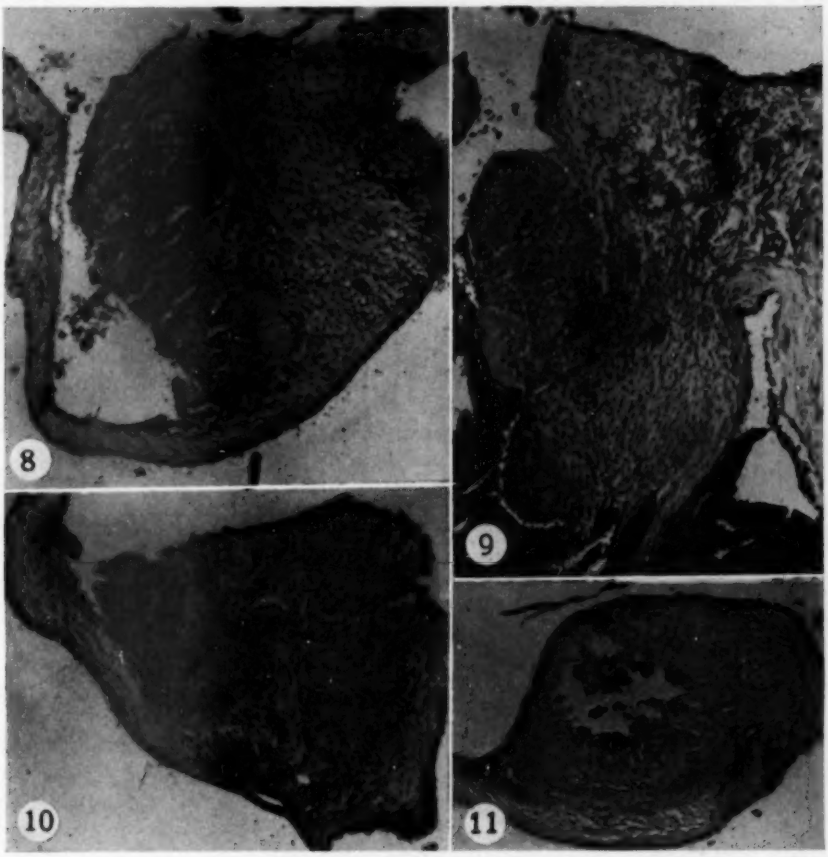


Fig. 8 (Rat 819).—Mitral leaflet at 96 hours after inoculation of Str. faecalis and 24 hours after beginning of treatment. In left upper quadrant at base of vegetation is dark round mass of leucocytes with a few bacteria. Note beginning organization of vegetation. Hematoxylin azure eosinate; × 65.

Fig. 9 (Rat 482).—Mitral leaflet five days after inoculation of Str. faecalis and four days after beginning of penicillin therapy. Organization is nearly complete except for small area below center along left border. Note wide separation of cells in pale area in upper leaflet. A few vessels are seen at base of leaflet. Hematoxylin azure eosinate; × 60.

Fig. 10 (Rat 608).—Mitral leaflet 11 days after inoculation of Str. faecalis and six days after beginning of treatment. Note endothelial-lined channels along ventricular surface at base of vegetation, which is nearly completely organized. Hematoxylin azure eosinate; × 65.

Fig. 11 (Rat 1022).—Mitral leaflet 16 days after first and seven days after last of five inoculations with Str. mitis. Note area of necrosis and calcification in center of organized vegetation. Hematoxylin azure eosinate; × 65.

by bacteriolysis and phagocytosis. Bacteriolysis was characterized by gradual loss of basophilia and other staining characteristics of the colony. The colony became converted into a faint basophilic smudge and finally disappeared or was engulfed by converging phagocytes (Figs. 12 and 13). Occasionally, a few isolated colonies or a few bacteria within the colony or along its periphery retained their structural and staining characteristics even after treatment for eight days. Rarely, calcification ensued.

Macrophages were seen in many of the larger fading colonies. The outline of some of the smaller fading colonies corresponded to those of single or small groups of swollen macrophages, indicating that the bacteria were largely intracellular. At the base of vegetations, most of the bacterial colonies, some showing bacteriolysis, were surrounded and often disorganized by invading neutrophiles and macrophages (Fig. 13). Many of these cells contained Gram-positive bacteria, while other cells were fragmented. The chromatin derived from such cells coalesced to form a dense network of Feulgen-positive material along the border of some of the invaded colonies. The dark line along the lower border of the abscess in Figure 12 consists of such material.

Organization of Vegetations.—Organization of vegetations usually proceeded from the base to the free surface of the vegetation. It was accomplished by an ingrowth of fibroblasts from the underlying valvular stroma. In the earlier stages of organization, the invading fibroblasts were intermingled with and preceded by leucocytes and macrophages, some containing bacteria and nuclear debris. Many of these phagocytes were surrounded by a clear space, suggesting that they play a role in the resolution of the matrix of the vegetation. Even large vegetations were often thoroughly permeated by streamers of fibroblasts within 48 hours after the beginning of delayed therapy and nearly completely organized within six days (Fig. 10). However, little or no resolution and organization of the vegetation were noted in the vicinity of persistent colonies resisting therapy.

Encapsulated Vegetations.—The free surface of some vegetations, particularly smaller vegetations and peninsular portions of large vegetations, was covered at an early period (sometimes within 48 hours after the beginning of therapy) wholly or in part by a layer of endothelium and one or more underlying layers of fibroblasts (Figs. 12, 13, and 14). In early stages (24 to 48 hours after the beginning of delayed therapy), the unorganized portion of such vegetations often contained colonies undergoing bacteriolysis or phagocytosis (Fig. 13). In later stages, however, no bacteria were observed, and the unorganized central portion often appeared partially hyalinized (Fig. 14). Such encapsulated lesions without demonstrable bacteria were seen occasionally in leaflets showing bacteria-laden vegetations in other areas.

Changes in the Valves.—The inflammatory reaction in the leaflets subsided rapidly. Within 24 to 48 hours after the beginning of delayed therapy, there often were few or no leucocytes in the valvular stroma, even when numerous leucocytes were seen nearby around remnants of colonies at the base of vegetations. The increased cellularity and the other changes in the stroma previously described receded more slowly than the leucocytic infiltration (Figs. 8, 9, and 10). A small number of treated rats were killed several weeks after apparent recovery following a single bacterial inoculation. They presented no evidence of a preceding valvulitis or only minor changes, such as slight fibrous thickening of the leaflet and one or

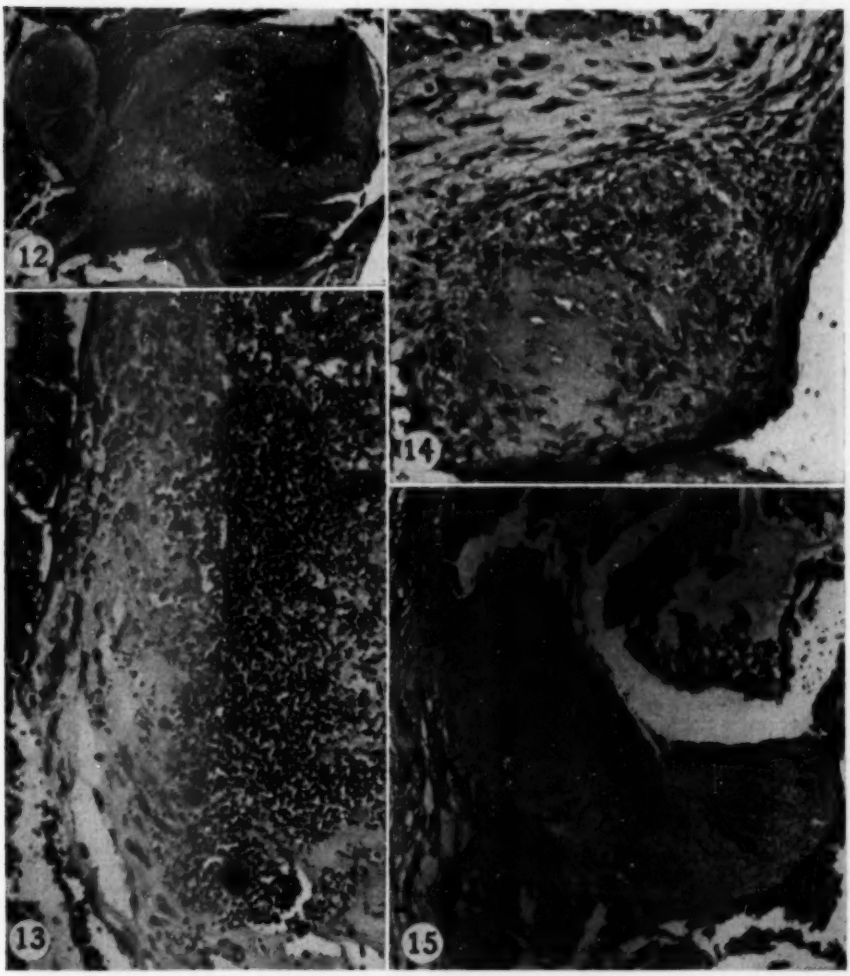


Fig. 12 (Rat 473).—Mitral leaflet 10 days after first of four inoculations with Str. faecalis and two days after beginning of treatment. In right upper quadrant is large abscess marginated below by a dark irregular line (see text). Along upper auricular surface immediately to left and above abscess is peninsula of unorganized portion of vegetation. This is shown at higher magnification in Figure 13. Hematoxylin azure eosinate; × 24.

Fig. 13 (Rat 473).—Peninsula of vegetation horizontally disposed in Figure 12. Auricular surface on left is covered by endothelium and one or more layers of fibroblasts, while base on right of figure is densely infiltrated by phagocytes. In lower midportion are three dark gray bacterial colonies partially surrounded by phagocytes. A cross section of peninsula might resemble vegetation in Figure 14. Hematoxylin azure eosinate; × 150.

Fig. 14 (Rat 798).—Encapsulated aortic vegetation six days after inoculation of Str. faecalis and three days after beginning of treatment. Phagocytes are seen at base of vegetation and in adjoining portions of leaflet. No bacteria are demonstrable. Hematoxylin azure eosinate; × 165.

Fig. 15 (Rat 1140).—Aortic leaflet five months after seven injections of Str. mitis and penicillin therapy. Note marked fibrous thickening and distortion of leaflet and dark calcium deposits on upper and lower surfaces of leaflet near junction with root of aorta above and thickened ventricular endocardium below. Hematoxylin azure eosinate; × 40.

more foci of mucoid degeneration or small collections of mononuclear cells. Similar changes, usually less marked, are seen occasionally in ground level rats and more frequently in altitude rats.

Valvular Lesions Following Multiple Bacterial Inoculations

After multiple inoculations the valvular lesions in untreated altitude rats usually developed more rapidly and were severer than after a single inoculation of the same micro-organism, but the pathologic features at a given stage of development were essentially similar to those described. The response to penicillin was less favorable. Organization of vegetations was relatively slow and was occasionally incomplete even after several months. Some of the organized vegetations contained one or more foci of necrosis, suppuration, hyalinization, calcification, or, rarely, chondrification (Fig. 11). Animals killed after apparent recovery from the acute infection often revealed marked irregular thickening and deformity of one or more leaflets. Some leaflets showed areas of calcification, hyalinization, and other degenerative changes or adherence to the aorta or mural endocardium (Fig. 15).

Endothelial-lined spaces containing blood were seen in some thickened leaflets (Figs. 9, 10, 11, and 15). Some near the base of the leaflets were unequivocal blood vessels, probably communicating with vessels in the valvular ring. After study of skip serial sections, it was concluded that some channels communicated with the ventricular cavity and were sections of endothelialized fissures or clefts in organized vegetations or spaces between folds in the leaflet. The origin of other channels was not determinable.

Valvular Lesions in Inoculated Ground Level Rats

Endocarditis developed in a relatively small number of inoculated control rats maintained at ground level.¶ The valvular lesions were essentially similar to those described in altitude rats, but were usually less severe and slower in development. A few rats, killed after receiving 12 bacterial injections in three weeks, showed marked cellular proliferation in a leaflet without demonstrable vegetations. These changes were considered to be due to a mild or receding infection, since similar valvular changes were seen in infected leaflets near organizing small bacterial vegetations.

Myocardial Lesions in Altitude Rats Following Single and Multiple Bacterial Inoculations

The incidence and severity of myocardial lesions varied considerably in different groups of animals but tended to increase with the number of bacterial inoculations and the length of survival. Among the commoner lesions were abscesses in the region of the valvular ring, focal interstitial myocarditis, and bacterial emboli in coronary branches associated at times with infarcts.

Lesions in the Valvular Rings and Adjoining Myocardium.—The commonest complication of valvular endocarditis was the development of inflammatory changes in and near the valvular rings. The earliest change, noted 20 hours after inoculation, consisted of congestion and a slight infiltration of the valvular ring by neutrophiles. In some instances, the inflammatory focus was connected with a similar lesion in the adjoining leaflet by a narrow subendothelial bridge of leucocytes; the bridge

¶ References 1 and 2.

skirted below the root of the aorta when joining an aortic lesion. After the first day, the inflammatory reaction increased in severity and extended into the adjoining trigone, the interstitial tissue of the adjoining myocardium, and occasionally the epicardium. The inflammatory area showed a dense infiltration by neutrophiles and mononuclear cells, swelling and proliferation of fibroblasts with occasional mitotic figures, some Anitschkow myocytes, occasional hemorrhagic extravasations, and atrophy or necrosis of some muscle fibers. The intercellular matrix often showed edema and abundant material staining like acid mucopolysaccharides. After the fourth day, some animals showed evidence of subsiding reaction, while others, particularly among those receiving multiple inoculations, showed abscess formation

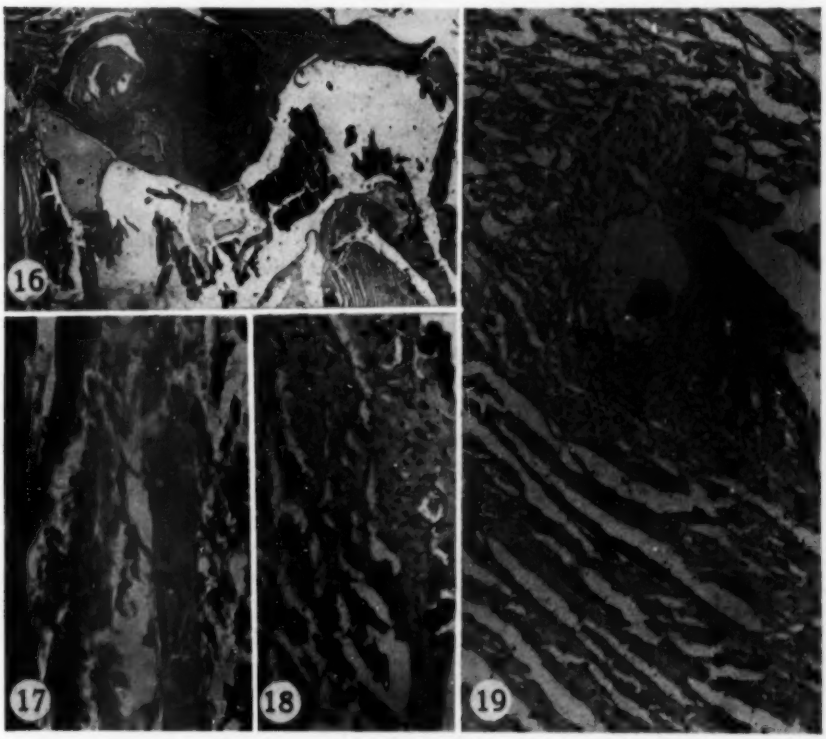


Fig. 16 (Rat 414).—Periaortic abscess to right of aorta (in left upper quadrant) in untreated rat killed two weeks after first of eight injections of Str. mitis. Note that mitral leaflets are normal, while aortic vegetations occupy much of ascending aorta. Hematoxylin azure eosinate; × 10.

Fig. 17 (Rat 574).—Myocardium of rat killed four days after single inoculation of Str. faecalis, showing focal interstitial myocarditis as described in text. Hematoxylin azure eosinate; × 325.

Fig. 18 (Rat 819).—Same rat as in Figure 8, showing small groups of necrotic calcified muscle fibers, which are scattered throughout ventricular myocardium. Hematoxylin azure eosinate; × 40.

Fig. 19 (Rat 464).—Myocardium two days after inoculation of Str. faecalis, showing bacterial embolus in coronary branch. Below artery is small triangular infarct (indistinct in figure) with apex approaching right lower corner of figure. Hematoxylin azure eosinate; × 95.

in the trigone (Fig. 16). A few abscesses formed huge protruding masses occupying most of the space in the ascending aorta or left auricle. The abscesses often showed marked fibroblast proliferation along their periphery and rarely contained bacteria or calcareous deposits. In some instances after treatment the abscess was replaced largely by granulation tissue infiltrated by a variable number of leucocytes. In other treated animals, there were scars suggesting healed abscesses (Fig. 15).

Focal Interstitial Myocarditis.—These lesions were noted in nearly half the animals that died or were killed after the fourth day following inoculation in a few experimental groups. They were found only rarely in other groups or before the fourth day. The lesions were scattered throughout the ventricular myocardium, varied markedly in size and shape, and consisted chiefly of young fibroblasts admixed with large mononuclear cells and lymphoid cells (Fig. 17). Many lesions contained one or more necrotic or calcified muscle fibers. With the periodic acid-Schiff procedure, the necrotic fibers stained deep red. Giant cells, as described in Brächt-Wächter bodies,[11] were not encountered, though cells with two or more nuclei were seen occasionally. No bacteria were found. The intercellular ground substance contained material staining like acid mucopolysaccharides. A few lesions slightly resembled but were not considered typical of Aschoff bodies.

Calcified Lesions.—A few animals examined on the fourth day following an inoculation showed scattered groups of calcified ventricular muscle fibers with little or no surrounding cellular reaction (Fig. 18). The necrotic fibers were stained by the periodic acid-Schiff procedure. Vessels in these lesions occasionally showed calcified plaques in the intima or media. The distribution of the lesions and their size were essentially similar to those described in animals with focal interstitial myocarditis. No bacteria were found in the lesions.

Vascular Lesions.—Bacterial emboli were seen in one or more coronary branches as early as the second day following inoculation. They were associated with a marked leucocytic reaction involving the wall of the artery and its immediate surroundings and occasionally with small myocardial infarcts (Fig. 19). Occasionally, no bacteria were demonstrable in such emboli. After the second week, there were occasionally seen patent arterioles showing a dense infiltration of the wall and surrounding myocardium by neutrophils and fewer mononuclear cells. Sometimes there were areas of interstitial fibrosis or scarring in the myocardium suggesting healed infarcts. In some instances, a few vessels showed minor changes, such as focal hyalin degeneration of the wall and margination by a few mononuclear cells.

Other Lesions.—Even in relatively mild infections of the aortic leaflets, the adventitia of the ascending aorta and the surrounding fatty tissue were often densely infiltrated, chiefly by large mononuclear cells. In severe infections, the root of the aorta was disorganized and densely infiltrated by neutrophiles. The base of the aorta was frequently involved by extension of vegetative lesions. Occasionally, the ascending aorta near the arch showed a bacterial vegetation or necrosis with extensive ulceration and occasionally perforation.

Other lesions noted occasionally included organizing sterile thrombi in the auricles, focal infiltration of the epicardium or mural endocardium by mononuclear cells and sometimes neutrophils, and nondescript patches of fibrosis and hyalinization, particularly in and adjoining the valvular rings and the papillary muscles of the left ventricle. In addition to the changes listed, cardiac hypertrophy and other changes attributable to exposure to high altitude were noted.[7]

COMMENT

Our findings in untreated inoculated altitude rats are not unlike those described in acute bacterial endocarditis in man # and in experimental endocarditis induced in rabbits by MacNeal and his associates.*

Clawson † has studied endocarditis in normal rats following one to three intracardiac injections of viridans streptococci and Streptococcus pyogenes. In one study, he found bacterial endocarditis in 12.4% and rheumatic-like endocarditis in 18.9%. He considered the two lesions anatomically similar to the two types commonly found together in the same valve in cases of subacute bacterial endocarditis, but stated that the rheumatic-like vegetations in the rat were not necessarily etiologically the same as the lesions found in human valvulitis. Some of the rheumatic-like vegetations described and illustrated by Clawson resemble the encapsulated vegetations without demonstrable bacteria described in the section on regressive changes (Fig. 14). We consider such encapsulated lesions to be derived from bacterial vegetations, since bacteria were found in otherwise similar lesions in altitude rats (Fig. 13). These findings suggest that some of the nonbacterial lesions seen in human endocarditis may be derived similarly from bacterial lesions.

It seems important to emphasize the remarkably short period required for the development and regression of large bacterial vegetations even in untreated rats. Two or three weeks after inoculation, an area of increased cellularity and thickening may be the only evidence of a preceding bacterial vegetation in a leaflet. On the other hand, certain bacterial colonies may persist despite penicillin therapy, and their continued presence prevents or impairs the resolution and organization of the neighboring portion of the vegetation. Such colonies may be destroyed eventually. However, their previous presence may account for the foci of necrosis, calcification, or impaired resolution and organization seen in some vegetations in rats recovering after severe infections (Figs. 11 and 15). Since these foci may be surrounded by abundant fibrous tissue resulting from organization of the remainder of the vegetation (Fig. 11), they may be difficult to differentiate from possible foci of necrosis or degeneration in the valvular stroma.

The origin of bacterial vegetations has been the subject of some controversy.‡ According to Allen,§ vegetations in human endocarditis are derived principally from necrotic valvular tissue forced apart by the plasma and blood elements exuded by the eroded or abnormally permeable blood vessels found in the inflamed valves. It must be pointed out, however, that the inflamed leaflets in the experimental rats, unlike those in human endocarditis, generally have no demonstrable vessels adjoining vegetations. Moreover, material staining for acid mucopolysaccharide was not seen even in the matrix of young vegetations overlying inflamed portions of a leaflet containing an abundance of such material. This fact suggests that the matrix of vegetations in the experimental animals contain little or no material derived from the intercellular ground substance of the inflamed leaflet. The endocardial vegetations of altitude rats occasionally contained detached or partially detached

References 11 and 12.

* References 13, 14, and 15.

† References 16, 17, and 18.

‡ References 11 and 12.

§ References 19 and 20.

portions of the superficial stroma of the inflamed leaflet. However, these fragments constituted only a small portion of the vegetation and differed morphologically and tinctorially from the surrounding matrix.

Our findings in early infections are more consistent with the view [11] that vegetations are largely thrombi formed by deposits from the blood on an inflamed endocardial surface. In early infections, the matrix of the vegetations in the experimental rats closely resembles fibrin. In later infections, however, it is less typical morphologically and tinctorially, suggesting physical or chemical alterations or possible admixture with other elements derived from either the blood or valvular stroma. Older vegetations show numerous small clefts. These may be considered to be due to various causes, such as circulatory stress, cellular enzymatic activity (evidenced by clear spaces around phagocytes), or contraction of fibrin with age, indicated microscopically by a more compact structure. Some clefts contained blood and were found by studying skip serial sections to communicate, often by a devious channel, with the outer surface. Other clefts appeared empty and may be presumed to have contained uncoagulated fluid. Such fluid may be a product of physical or chemical (including enzymatic) processes occurring in the surrounding matrix of the vegetation, or the fluid may be derived by seepage through the matrix of the vegetation directly from the blood stream or indirectly after passing through the inflamed leaflet. The presence of uncoagulated fluid in the inflamed leaflet is evidenced by the occurrence of irregular clear spaces between some of the cells (Fig. 1). Such fluid seeping in from the leaflet could conceivably carry into crevices disorganized fragments of valvular stroma or of elastic and argyrophilic fibrils such as are seen occasionally in unorganized areas of the vegetation. Since the composition of fluids in the living organism is subject constantly to change, it seems likely that in the course of time some of the clefts in the vegetation would be obliterated by coagulation of their fluid contents, while new clefts would be formed elsewhere. Such a dynamic process would account for the varied morphological and tinctorial changes occurring in the vegetations.

The structural details of bacterial colonies in the vegetation were often obscured by the density of the bacteria. However, after the onset of bacteriolysis, it was noted that many of the smaller colonies lay within single or small clusters of large swollen mononuclear cells, some showing karyolysis. A number of the larger colonies contained similar cells (Fig. 5). The gradation between bacteria-laden macrophages and satellite colonies suggests that some of the latter may be derived from macrophages overwhelmed by the bacteria they have ingested (Figs. 5 and 7). These findings suggest that bacteria ingested by large mononuclear cells and possibly endothelial cells (Figs. 3 and 4) may in some instances continue to survive or proliferate without causing immediate death of the cells. If the cells are finally destroyed, others may attempt to invade or wall off the colony.

The bacteria-laden macrophages may help to explain the frequent recurrences following premature discontinuance of antibiotic therapy in endocarditis. It has been shown that the bactericidal action of penicillin both in vitro [21] and in vivo [22] is paradoxically reduced when the organisms are not metabolizing and multiplying at their optimal rate. To effect cure in such cases may require a more prolonged period of treatment with penicillin or the concomitant use of an antibiotic that is effective even against organisms which are not actively metabolizing. It is now known that penicillin diffuses freely into mammalian cells.[23] However, since it

may be presumed that the bacteria in the macrophages are not metabolizing and multiplying at a maximum rate, it may be assumed that they are relatively resistant to penicillin. Doubtless many of the macrophages eventually destroy all the bacteria they have ingested, but some macrophages are overwhelmed by the bacteria (Fig. 5). Such bacteria may then begin to metabolize more actively and become sensitive to penicillin if the penicillin is present in sufficient concentration. A few organisms, however, may escape for a time by being engulfed by new macrophages. Such a cyclical process would help to explain the need for and frequent effectiveness of prolonged therapy.

Another possible factor reducing the effectiveness of penicillin is the dense matrix surrounding many of the colonies in the vegetations. It may impede the diffusion both of the antibiotic and of the nutrilites; this factor would limit the growth of the micro-organisms, thereby increasing their resistance to penicillin. The occurence of bacteria in foci of suppuration and necrosis in the kidney || as well as in the heart would similarly limit the efficacy of penicillin. The remultiplication of a few surviving bacteria following cessation of treatment would explain apparent recurrences of the disease. Such remultiplication of surviving bacteria has been shown to occur in localized experimental infections in mice, and bacterial counts made on the heart and kidney of our experimental animals have shown that similar remultiplication occurs after premature cessation of treatment.[23]

The localization of the valvular ring abscesses in the altitude rats was similar to that found in man by Sheldon and Golden.[24] They suggested a relationship of such abscesses to antibiotic therapy because of the high incidence of such abscesses in patients with acute bacterial endocarditis who had received antibiotic therapy. In man, the abscesses appeared to arise from mycotic aneurysms in the valve rings. In altitude rats, study of early infections suggested a relationship of such abscesses to severe lesions in adjacent valvular leaflets. The abscesses developed later and more slowly than the vegetations, tending to increase for a limited period with the age of infection. They responded more slowly to therapy. We found a relatively high incidence of abscesses in rats dying after institution of therapy. This may have been due to the prolongation of life in animals with fulminating infections, which permitted more time for suppuration to develop even while some vegetations were regressing. The high incidence of such abscesses in human endocarditis following antibiotic therapy may be explained in a similar manner.

The calcified myocardial lesions (Fig. 18) in our infected animals did not contain bacteria and closely resembled similar lesions produced experimentally in mice by a purified low-protein diet [25] and in rats by a potassium-deficient diet.¶ They were not unlike the calcified myocardial lesions described at times in diphtheria.[11] These facts suggest that the lesions are due to an acute toxic, nutritional, or metabolic disturbance precipitated by the infection. It is suggested that the interstitial cellular foci (Fig. 17), some of which include one or more similarly calcified muscle fibers, are etiologically related to the calcified lesions. The difference may be due to a cellular reaction to the necrotic fibers. Healing of such foci may explain some of the myocardial scars seen occasionally after recovery.

|| References 3 and 4.

¶ References 26 and 27.

SUMMARY AND CONCLUSIONS

Experimental bacterial endocarditis was produced regularly in rats acclimatized to a simulated altitude of 25,000 ft. by a single intravenous injection of 0.5 cc. of a six-hour broth culture of Streptococcus faecalis. The animals showed bacterial vegetations and nonbacterial lesions (without demonstrable bacteria) resembling similar lesions seen in human endocarditis. The development of the valvular lesions and their regression following penicillin therapy were studied in detail.

The vegetations are considered to arise as thrombi formed by deposits from the blood on an inflamed endocardial surface. Subsequent changes are described and discussed.

The bacterial colonies in the valvular vegetations consisted in part of bacterial-laden macrophages. Experimental evidence indicates that bacteria in such macrophages are relatively resistant to penicillin and may be an important factor in recurrences following premature cessation of treatment.

Nonbacterial lesions may be derived from bacterial vegetations. This situation results, after destruction of bacteria by phagocytosis and bacteriolysis, when the external surface of the vegetation becomes covered by endothelium and fibrous tissue before the central portion of the vegetation is organized.

The histologic appearance of valvular ring abscesses, bacterial emboli, infarcts, interstitial myocarditis, and other myocardial lesions associated with experimental endocarditis is described and their significance is discussed.

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PHYSIOLOGIC AND HISTOCHEMICAL CHANGES IN CONNECTIVE TISSUE OF RAT INDUCED BY TOTAL BODY IRRADIATION

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WHOLE-BODY ionizing irradiation has been observed to increase vascular permeability,[1] which may be correlated with radiation-induced capillary fragility and hemorrhage.[2] Irradiation has also been reported to enhance the permeability of the dermis to intradermally injected dye.[3] The pathogenesis of these alterations is not understood, but it is conceivable that the vascular changes and the disturbances of dermal permeability are related to one another. These studies were undertaken to correlate, if possible, these two radiation-induced permeability phenomena and to search for associated morphologic abnormalities.

MATERIALS AND METHODS

Wistar rats of both sexes, 10 to 14 weeks old, were divided into pairs of litter mates. One of each pair received 750 r of whole-body x-radiation, with the following factors: 250 kvp, 68 to 81 r per minute, 30 ma., 3 mm. of Al filtration, T.S.D. 90 cm.; the other rat was employed as a nonirradiated control. The animals of each pair were of the same sex and were fed and caged together under standard conditions.

After irradiation, 0.05 ml. of 0.5% Evans blue in distilled water was injected intradermally into irradiated and control rats in corresponding areas of dorsal skin. Injections were begun on the day of irradiation and performed at intervals throughout the first 30 days after exposure. Only one test was performed per rat. Prior to injection the skin was shaved with an electric razor, and the animal was anesthetized to prevent bodily movement which might exert pressure on the wheal of dye. This was accomplished by pentobarbital sodium (Nembutal), injected subcutaneously in a site remote from that used for the spreading test. Measurements in millimeters of the longest diameter (D) and shortest diameter (d) of the elliptical dye-colored area of skin were made one, two, three, and four hours after injection, and the areas of spreading of discoloration were calculated from the data given by the formula $\frac{D \times d \times \pi}{4}$ (Hechter [4]).

Tissues for histological study were obtained from a separate series of male rats of the same age and strain, irradiated as described. On the 10th postirradiation day 15 animals were killed (by luxation of the cervical vertebrae); 10 nonirradiated litter mates were simultaneously killed as controls. An autopsy was performed on each rat, and sections of skin were fixed with 95% ethyl alcohol, Zenker-formol (Helly's) solution, and freeze-drying. Paraffin sections of skin were stained with hematoxylin and eosin, aqueous toluidine blue (1:100,000), a modification* of the Hale[5] reaction for acid polysaccharaide, and the periodic acid-Schiff routine. Selected sections were stained by the Giemsa method, Best's carmine stain, and the Feulgen reaction. Representa-

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Work performed under Contract W-7405-eng-26 for the Atomic Energy Commission.

* The concentration of potassium ferricyanide solution was reduced from the specified 0.02 M to 0.002 M, since this concentration was found to enhance the contrast between irradiated and control animals.

tive sections were also incubated in bovine testis hyaluronidase, 1.0 mg. in 1.0 ml. of 0.3% saline solution at 37 C. for varying intervals up to one hour, control sections being incubated similarly in 0.3% saline solution and in distilled water. In addition, representative sections were stained in varying dilutions of toluidine blue (1 : 10,000 to 1 : 100,000) in distilled water and in 10% to 70% alcohol.

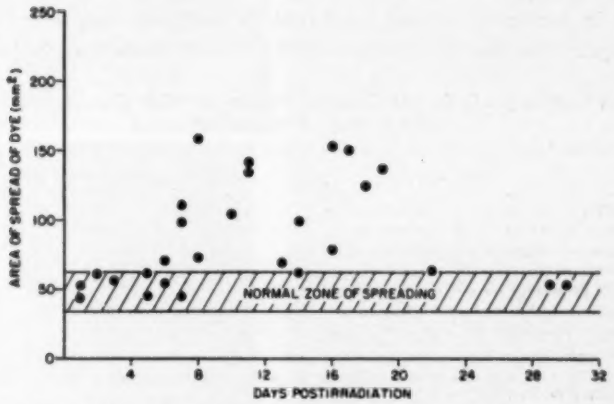

Fig. 1.—Dermal spreading of Evans blue in male rats after x-irradiation. Each point represents area of spreading of 0.05 ml. of 0.5% Evans blue in distilled water four hours after intradermal injection. A separate rat was used to determine each point.

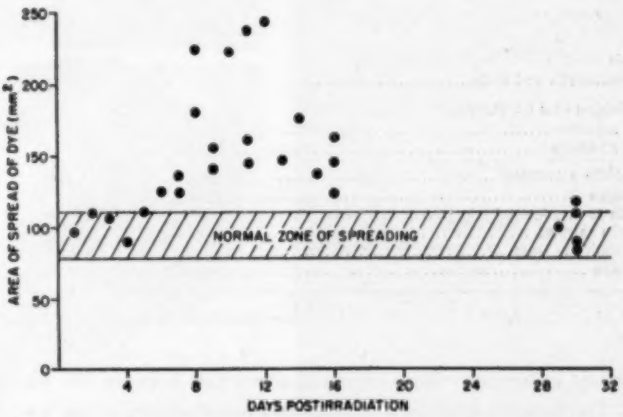

Fig. 2.—Dermal spreading of Evans blue in female rats after x-irradiation. Each point represents area of spreading of 0.05 ml. of 0.5% Evans blue in distilled water four hours after intradermal injection. A separate rat was used to determine each point.

RESULTS

The spreading of dye in the dermis of normal rats conformed to the pattern described previously.[6] The extent and variability of spreading were greater in females than in males, as has been reported.[7] Until the fifth day after irradiation the diffusion of dye in the irradiated rats did not differ significantly from that in the nonexposed controls; after five to seven days, however, the dye spread

much more rapidly and more widely in the irradiated animals. This difference persisted for 1 to 2 weeks, followed by recovery within 30 days (Figs. 1 and 2).

Histochemical findings in sections of skin, summarized in Table 1, include an excess of demonstrable intercellular material, staining with the Hale reaction for acid polysaccharide, located in the dermis and panniculus (Fig. 3, *a-e*), and diminution in number of dermal mast cells in irradiated rats. The intercellular material appeared as fine blue granules with the Hale reaction, distributed on and

TABLE 1.—*Changes in Dermal Ground Substance and Mast Cells of Rats After Whole-Body X-Irradiation**

	Irradiated Rats	Nonirradiated Rats
Number	15	10
Dose of x-rays	700 r	0
Amount of stainable ground substance		
In dermis	+++	+
In panniculus	+++	+
Relative no. of dermal mast cells	++	+++
Relative granularity of dermal mast cells	++	++

* Relative amounts of stainable ground substance and numbers and granularity of mast cells are graded semiquantitatively 0 to ++++.

TABLE 2.—*Histochemical Properties of Ground Substances and Mast Cells in Dermis of Irradiated Rats*

	Results *	
Methods	Ground Substance	Mast Cells
Hematoxylin and eosin	—	—
Toluidine blue (1:100,000)		
Aqueous	—	+++
Alcoholic	—	+++
Periodic acid-Schiff	—	+++
Feulgen	—	—
Hale (acid polysaccharide)	+++	+++
Best's carmine	—	—
Solubility in water	+++	—
Giemsa	—	+++

* Intensity of positive staining reaction is graded semiquantitatively + to +++; a negative reaction is denoted by —.

between dermal connective tissue cells and fibers and between the fat cells of the panniculus. The contrast between irradiated and nonirradiated animals was enhanced by using the modified Hale procedure, as described, and examining the sections under oil immersion: In nonirradiated animals little Hale-positive material was discernible, whereas in irradiated rats the granular substance was abundant.

Histochemical properties of the material are summarized in Table 2. This intercellular ground substance was best demonstrated in tissue fixed in 95% alcohol or by freeze-drying; fixation in Zenker-formol solution (Helly's) or immersion of sections in other aqueous solutions resulted in a diminution of stainable intercellular material. Incubation of sections with hyaluronidase gave little information, since there was essentially equal loss of intercellular substance in control sections

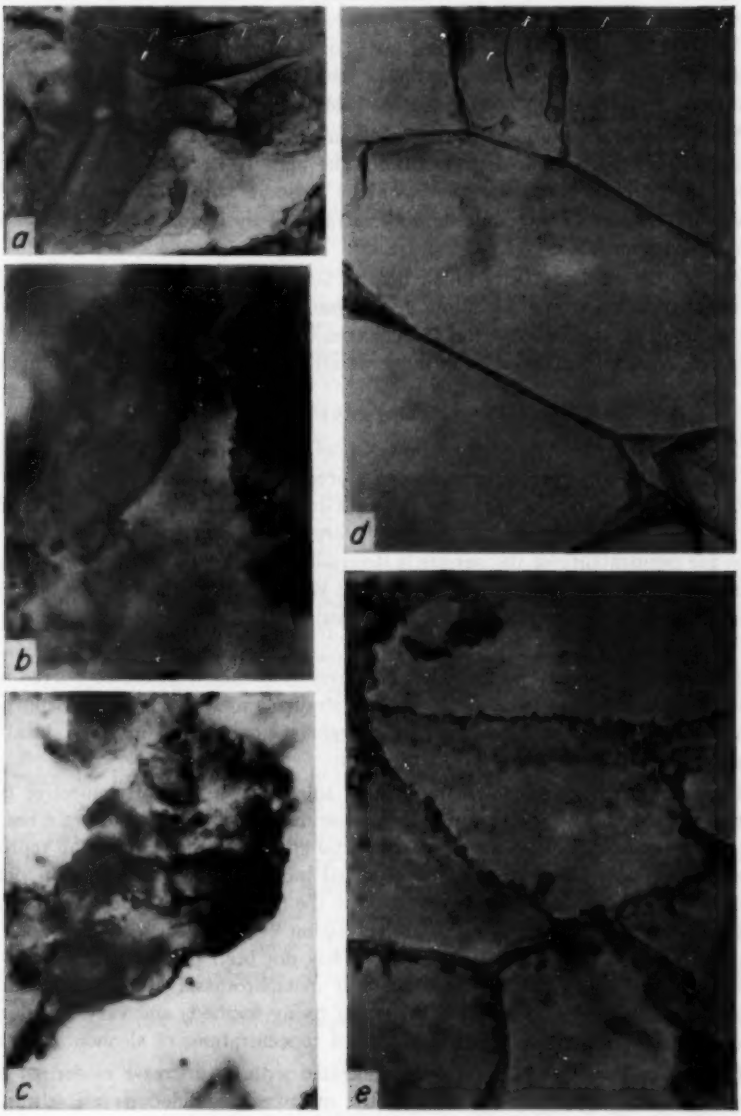

Fig. 3.—(*a*) Dermal collagen fibers of a nonirradiated rat; interfibrillar ground substance is inconspicuous. Modified Hale-hematoxylin technique; × 775. (*b, c*) Dermal collagen fibers of an irradiated rat; granular ground substance is conspicuous at edges of fibers. Modified Hale-hematoxylin technique; × 775. (*d*) Subcutaneous adipose tissue of a nonirradiated rat; intercellular ground substance is inconspicuous. Modified Hale-hematoxylin technique; × 775. (*e*) Subcutaneous adipose tissue of an irradiated rat; granular ground substance is conspicuous between fat cells. Modified Hale-hematoxylin technique; × 775.

incubated in saline alone or in water. Attempts to demonstrate a metachromatic reaction in Clarite-mounted sections after staining with dilute toulidine blue (1 : 10,000 to 1 : 100,000) in distilled water or in 10% to 70% alcohol were unsuccessful, even though after being removed from the toluidine blue the sections still contained intercellular substance demonstrable with the Hale reaction.

Reduction in the numbers of mast cells in the dermis of irradiated rats was not accompanied by detectable degranulation. The mast cell granules stained intensely blue with the Hale reaction, violet with the periodic acid-Schiff method, and red with toluidine blue. Their staining was not altered by irradiation or by treatment with hyaluronidase.

All of the irradiated rats killed on the 10th postirradiation day revealed the acute radiation syndrome, including generalized hemorrhage into the lymph nodes. The sections of skin, except for the changes in mast cells and ground substance described, were essentially alike in the irradiated and the nonirradiated animals.

COMMENT

The increase in dermal permeability simulated, in time of onset, progression, and duration, the hemorrhagic tendency induced by irradiation and measured quantitatively earlier in rats of the same stock.[8] Thus it would appear that the capillary wall and the connective tissue of the dermis are rendered permeable simultaneously. Since the permeability of the dermis is determined largely by the ground substance of dermal connective tissue [9] and that of the vascular membrane by the interendothelial cement,[10] one may speculate that irradiation alters these intercellular materials in a like manner.

The increased dermal permeability observed by earlier workers [8] occurred much sooner after irradiation than in the present studies and was unrelated to purpura; however, unlike this investigation, the previous experiments involved local and massive, rather than whole-body, irradiation.

The nature of the change in the ground substance of dermal connective tissue is not yet known. The increase in polysaccharide stainable with the Hale reaction does not necessarily indicate an increase in total ground substance but may represent merely a chemical alteration. Sylvén reported increased metachromasia of dermal connective tissue, associated with reduction in the number of perivascular mast cells in the dermis of the rat, after local irradiation.[11] While such observations are comparable to the findings of this study, it has not been possible thus far in this laboratory to confirm the increased dermal metachromasia after either local or whole-body irradiation by use of a variety of fixing methods and varying dilutions of toluidine blue in distilled water and graded concentrations of alcohol.

The reduced number of mast cells associated with the increase in dermal polysaccharide led Sylvén to postulate that the mast cell extruded its metachromatic material into the surrounding tissue.[11] This has been reported to occur also in irradiated human skin.[12] Such a "secretion" of heparinoid material by the mast cell has been regarded as a possible explanation for the hemorrhagic effects of irradiation.[13] In the present study, extrusion of mast cell granules was not definitely demonstrable, although vacuolation of their cytoplasm after irradiation was noted, as observed earlier.[14] Since the ground substance does not react metachromatically with toluidine blue and is soluble in water, it is sharply distinguishable

from the mast cell granules. Thus, from available evidence it cannot be concluded without reservation that the mast cells were the source of the increased stainable acid polysaccharide.

The possibility of a direct action of radiation on intercellular material is suggested by the experiments of Schoenberg and associates.[15] These investigators noted reduction in the viscosity and average molecular weight of hyaluronate massively irradiated in vitro. Likewise, depolymerization of intercellular glycoprotein of the lens of the eye is suggested by histochemical studies of radiation-induced cataract.[16] Similar effects on dermal ground substance have been postulated by Edgerly.[17] It is probable that such changes in ground substance contribute significantly to the susceptibility to bacterial invasion characteristic of the acute radiation syndrome.

Attempts to discover morphologic changes in interendothelial cement analagous to the radiation-induced effects in ground substance have been unsuccessful thus far; however, in addition to corresponding alterations of permeability, the thromboplastic activity of hyaluronate [18] and the susceptibility of blood platelets to inactivation by hyaluronidase [19] suggest that the capillary wall and the ground substance are physiologically related. One might speculate that the thromboplastic activity of the platelet is dependent on contained polysaccharide having hyaluronate-like properties and that this material contributes to the maintenance of vascular integrity, as suggested by the antihemorrhagic effects of platelet replacement perfusion in irradiated animals.[20] Rat platelets have been observed to contain material staining as acid polysaccharide with the periodic acid-Schiff and Hale techniques and with toluidine blue.† It is of further interest that heparin is believed to be an acid polysaccharide also, probably a sulfonated hyaluronate, and possibly derived from mast cells.[21] It is conceivable that the permeability of the interendothelial cement may be antagonistically influenced by blood platelets and by mast cells. This hypothesis deserves further investigation.

SUMMARY

Evans blue injected intradermally in rats after whole-body x-irradiation spreads more rapidly and more widely than in nonirradiated litter mates.

The radiation-induced permeability of dermal connective tissue appears four to seven days after irradiation and persists for from two to three weeks, the period coinciding with that of increased vascular permeability and purpura.

The heightened permeability of the dermis is associated with increased stainable acid polysaccharide in the ground substance of the dermis and panniculus, as indicated by the Hale stain, and with reduction in the number of mast cells in the dermis.

† Upton, A. C., and Gude, W. D.: Unpublished data.

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Obituaries

COL. VIRGIL HEATH CORNELL
1890-1954

COLONEL VIRGIL HEATH CORNELL, United States Army Medical Corps, Retired, died suddenly, June 3, 1954, as result of an acute coronary occlusion. Funeral services were held at 1:30 p.m., June 7, 1954, at Walter Reed Army Medical Center Chapel, with burial at Arlington National Cemetery.

Col. Virgil Heath Cornell, United States Army Medical Corps, Retired, was born in Brooklyn, on Nov. 29, 1890, son of Peter C. and Mary E. (Scholl) Cornell.

COL. VIRGIL HEATH CORNELL
1890-1954

He was married to Ruby C. Carpenter in 1916. Colonel Cornell attended Long Island College of Medicine, where he was graduated and received his M. D. Degree in 1913. Until he entered the Medical Corps of the United States Army in 1917, he served as assistant physician and pathologist at the Essex County Hospital, Cedar Grove, N. J. Colonel Cornell received the Degree of Doctor of Public Health from Harvard University in 1943.

Colonel Cornell's military assignments included service as Pathologist and Chief of Laboratory Service, Ancon Hospital, Canal Zone, Letterman General Hospital, San Francisco, and Walter Reed Army Hospital, Washington, D. C., and as Curator, Army Medical Museum, now the Armed Forces Institute of Pathology, from 1933 to 1935. During World War II, Colonel Cornell served as Command-

ing Officer of the 15th Medical General Laboratory, 1943-1945. Prior to this command he was Director of the Caribbean Area Laboratory, 1941-1943. Colonel Cornell retired from the military service in 1950 and entered the private practice of pathology in Washington, D. C., and Alexandria, Va., after his retirement.

Colonel Cornell was a Fellow of The American Medical Association, Fellow of the American College of Physicians, Fellow of the College of American Pathologists, Member of the American Association of Pathology and Bacteriology, American Society of Tropical Medicine, American Public Health Association, International Association of Medical Museums, and local Medical and Pathologic Medical Societies of the District of Columbia and Alexandria, Va. He was certified a Specialist by the American Board of Pathology.

For meritorious service during World War II, Colonel Cornell was awarded the Legion of Merit. He was the recipient of the Sternberg Medal for outstanding ability at the Army Medical School in 1921.

Colonel Cornell was the author of numerous medical articles and contributions pertaining to pathologic anatomy and clinical pathology. He was an excellent teacher of pathology and a champion to the younger medical officers training in the fields of pathology.

After retiring from military service, Colonel Cornell was a member of the pathology staff of George Washington University School of Medicine and attained the academic rank of professor in 1953.

BALDUIN LUCKÉ, M.D.
1889-1954

DR. BALDUIN LUCKÉ, Professor of Pathology at the University of Pennsylvania School of Medicine, died on April 26, 1954, at his home in Bryn Mawr, Pa. Thus ended a career of remarkable achievement. So diverse were his interests in pathology and his contributions so important that it is appropriate at this time to review the chief events of his career.

During his lifetime, the center of interest in pathology shifted from structural changes to alterations in function. Dr. Lucké was one of those who led this change in viewpoint, without, however, abandoning his concern with morphological problems. Indeed, his knowledge of functional changes increased the depth of his understanding of structural alterations.

Dr. Lucké was born in Hesse, Germany. Coming to this country in youth, he graduated in medicine from the Medico-Chirurgical College of Philadelphia. After a residency at the Philadelphia General Hospital, he joined the pathological staff of the University of Pennsylvania School of Medicine in 1914 under Allen J. Smith and remained a member of this faculty for forty years, that is, for the rest of his life.

During the First World War he served in the Army Medical Corps and was stationed at Camp Zachary Taylor, Ky. There he made his first important contribution to pathology. At that time the influenza pandemic was killing thousands of soldiers. In the "dead house" he performed many autopsies on the victims and acquired a profound knowledge of the pathological anatomy of influenza. His reports, however, dealt chiefly with its etiology, and he emphasized the changing aspect of the bacterial flora at different stages of the pandemic. Thus, during the earlier months, Hemophilus influenzae predominated to such an extent that it was regarded by many as the specific cause of the disease. Later, as Dr. Lucké reported, nonhemolytic streptococci and pneumonococci largely replaced H. influenzae, and these were obviously secondary invaders, while toward the end of the pandemic hemolytic streptococci and staphylococci dominated the picture. These, as Dr. Lucké recognized, were responsible for most of the complications and produced those profound changes in the morphological picture so ably described in his reports. At this time viruses had not yet been established as the specific cause of the disease.

After his return to civilian life in Philadelphia, Dr. Lucké's chief interests began to switch from morphology and etiology to functional changes in normal and abnormal living cells. This interest was greatly stimulated by his contact with Dr. Merkel Jacobs and the Marine Biological Laboratory at Woods Hole, Mass. There he found material admirably adapted to such investigations—the ova of sea urchins and other echinoderms. These cells, as long as they are uninjured, act as practically perfect osmometers. Consequently in anisotonic solution, the product of cell volume and osmotic pressure is approximately constant if allowance is made for osmotically

inactive cell contents, and therefore they obey the laws of Boyle and van't Hoff. After injury of various kinds, or because of calcium deficiency, permeability increases, often greatly. These experiments emphasized the importance of electrolyte balance in maintaining normal cell permeability.

At the same time, Dr. Lucké participated in a series of investigations of phagocytosis by leucocytes and its relation to other defense mechanisms, such as bacterial agglutination and electric charge.

In these years, Dr. Lucké acquired a well-deserved reputation as a fine teacher, impressing both students and colleagues with his clarity of mind and thorough grasp of his subject. His style of lecturing was emphatic; he left no doubts as to his views, and made a durable impression on his hearers' memories. Every presentation was

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prepared with meticulous care, and this was especially true of the brilliant clinico-pathological conferences, in which he collaborated with Alfred Stengel and O. H. Perry Pepper.

In the 1930's commenced his investigations of neoplasms in cold-blooded animals, a field in which he became master. The inception was the discovery, in the laboratory of A. N. Richards, of a frog having a mass in the kidney. Dr. Lucké at once grasped the possible significance of this observation and its potentialities for research. These adenocarcinomas proved to be very common in the kidney of leopard frogs. They could be transmitted by ground and frozen tumor to the kidney only, hence are presumably caused by a virus.

In a series of investigations with this tumor, lasting over twenty years, the following results are judged most significant:

1. The types of growth are determined chiefly by mechanical factors; the tumor forms sheets on solid surfaces (iris), tubules when growing free in the anterior chamber.

2. Temperature affects tumor growth in the same way as it does in normal tissue, and the value of the temperature coefficient is high ($Q_{10} = 2.5$). Higher temperatures greatly increase metastasis. The wide range of temperatures at which the tumor will grow greatly aided this investigation.

3. Irradiation by roentgen rays affects the frog tumor much as it does mammalian tumors. Intraocular transplants were irradiated and subsequently observed directly with the slit lamp microscope (with the collaboration of Dr. Schlumberger) and photographed at intervals. By this means, two widely held concepts in radiology were tested experimentally and confirmed: (a) Much larger amounts of irradiation are required to produce a certain effect when given in divided doses than in a single dose; (b) incomplete regression of a neoplasm due to inadequate irradiation may lead to resumption of growth at an unusually rapid rate.

After the frog kidney carcinoma, many more neoplasms in cold-blooded vertebrates were investigated, often with the colloboration of Dr. Schlumberger. Tumors proved to be common in amphibians, fish, and reptiles, and are similar in structure to corresponding tumors in man. Thus neoplastic growth is a phenomenon common to all classes of vertebrates.

During the Second World War, Dr. Lucké was recalled to active service in the Army and became deputy director, under Colonel Ash, of the Army Institute of Pathology (later, the Armed Forces Institute of Pathology) in Washington, D. C. At the end of the war he was retired a colonel. So valuable were his services regarded that he was awarded the Order of Merit.

It was at the Armed Forces Institute that he investigated epidemic hepatitis, and this is the work for which he was most highly acclaimed. The high incidence of hepatitis, with considerable mortality, led to autopsy material from various service hospitals being sent to Washington, where it was reviewed by Dr. Lucké. The results were published in three remarkable papers. It had become increasingly likely that "acute catarrhal jaundice" and "idiopathic acute yellow atrophy" represented two extremes of a liver disease probably of inflammatory origin. Dr. Lucké showed that this was indeed true and that both terms were misnomers and should be dropped. It was his accomplishment finally to reveal the cardinal lesions of hepatitis with precise descriptions.

The lesions vary greatly with the severity of the disease. In patients that recover, restitution of the liver to normal is the rule, whereas in fatal cases, the liver is distorted by tumor-like growths of regenerating, inadequately functioning cells, or in fulminant cases, is reduced to a spongy framework infiltrated by inflammatory cells and distended with blood.

Also, during the war period, he made one of his most brilliant contributions to pathology, on the syndrome that he named "lower nephron nephrosis."

The "crush syndrome" had been known since the First World War. Dr. Lucké showed that the same changes in function—anuria, shock—and in structure—heme casts, venous thrombosis, but especially degenerative changes of the lower nephron

—were common to the crush syndrome, to burns, transfusions with incompatible bloods, heat stroke, blackwater fever, toxemia of pregnancy, and poisoning with various agents.

All have an initiating factor of destruction of tissue or blood. In all, the renal lesion is essentially the same: degeneration, often necrosis, limited to the distal segments of the tubules, with brown casts of some heme compound in the lower nephrons and the collecting tubules. It is appropriate to designate all cases exhibiting these renal disturbances by a single term, "lower nephron nephrosis," because of the characteristic and unique location of lesions.

After he returned to the University of Pennsylvania at the close of the war, he succeeded as Chairman of the Department of Pathology (1947). In these last years he was everywhere recognized as one of the leading pathologists of this country. Though in impaired health, his interest in experimental work actually intensified.

He returned to his earlier work on cellular permeability, but now in order to compare permeability of neoplastic cells with that of normal cells. In this work he used a photoelectric method devised by Dr. Parpart, who was at times his collaborator. The inherent difficulty in this problem is that permeability can at the present time be measured only in uniform suspensions of free-living cells. For tumor cells, Dr. Lucké had recourse to ascites tumors and to certain lymphomas, but for normal cells he was limited to erythrocytes and different kinds of leucocytes (especially lymphocytes). Using the exquisite method of Parpart's densimeter, he found the relative permeability of certain tumor cells to a series of polyhydric alcohols to follow the same order as in mouse erythrocytes. This work on permeability of tumor cells will be continued by other collaborators.

In the field of enzymology, Dr. Lucké had long been concerned with enzymes of tumors and of tumor-bearing animals. As tumors of cold-blooded animals resemble mammalian tumors in structure, might the resemblance not extend to their enzymes? In fact, liver catalase does decrease in the frog bearing a renal carcinoma, as it does in mammals bearing carcinoma, and Dr. Lucké found on injecting homogenates of frog tumor into normals that the tumor contains some substance that inhibits catalase. In his last public presentation, before the Society for Experimental Pathology, less than two weeks before his death, Dr. Lucké demonstrated, in beautifully planned experiments with ascites tumor cells, a positive correlation between total volume and a number of tumor cells, and reduction of liver catalase.

One other project had occupied his attention: preparing a fascicle on kidney tumors for the "Atlas of Tumor Pathology," issued by the Armed Forces Institute of Pathology. Dr. Schlumberger will complete this volume.

In summarizing Dr. Lucké's contributions to the literature, of which only a part, regarded as the most important, has been mentioned, the material obviously falls into two categories: the morphological investigations, such as those on influenza, hepatitis, and nephrosis, and experiments on living cells, normal, injured, or neoplastic. It is hard to say which group is more important. His approach to experimental work, his viewpoint, what he hoped to (and did) accomplish, is much the same throughout the years; he was interested in seeing how well the gas laws hold in living material; he was interested in ascertaining whether neoplasms obey the

same laws of growth as do normal tissues and cells (he strongly opposed the view that tumors are "lawless" growths); he was interested in similarities between tumors in cold-blooded animals and those in mammals. In other words, he tried to find unifying principles in nature. He chose his problems deductively, proceeding from established principles to their application in certain material, and under certain experimental conditions. In effect he bound physics, chemistry, physiology, and pathology more closely together. He protested vigorously against the narrow concept that pathology means only, or even chiefly, structural change—it means not only that but includes etiology, pathogenesis, and abnormal function. This conception of pathology he stamped ineradicably on the minds of his colleagues and his students.

In personal relations, Dr. Lucké was notable for his limitless loyalty and devotion to the members of his staff and for his unselfish friendship to many others whose professional careers he greatly advanced. He possessed such a rare combination of abilities as to achieve excellence in teaching, investigation, and administration. By Dr. Lucké's death, we as pathologists have lost one of our most eminent colleagues, and a deeply mourned friend.

MORTON MCCUTCHEON, M. D.

News and Comment

American Board of Pathology Changes in Requirements for Certification in Special Fields of Pathology.—At a recent meeting the Trustees of the American Board of Pathology adopted the following requirements for certification in special fields of pathology, effective July 1, 1955.

Candidates who have met all general requirements and have had special training and experience which is acceptable to the Board in a special field of pathologic anatomy or clinical pathology may apply to the Board for certification in that special field. The Board, at its discretion, may approve this application, and after the candidate has successfully passed a prescribed examination or has fulfilled certain special qualifications (see below Section D), will issue a certificate designating the special field.

A. General Qualifications

1. Satisfactory moral and ethical standing in the profession.
2. License to practice medicine or a certificate of the National Board of Medical Examiners.
3. The applicant must devote his time primarily and principally to the practice of pathology or the special field of pathology in which he is requesting certification.

B. Professional Education

1. Graduation from a medical school in the United States approved by the Council on Medical Education and Hospitals of the American Medical Association or graduation from a medical school in other countries acceptable to the Board.

C. Special Training and Experience

The Board admits candidates to examination in special fields of pathology who are otherwise eligible and who have had either of the following types of training:

1. Applicants already holding a certificate of the Board in clinical pathology or the combined certificate in pathologic anatomy and clinical pathology (for qualification in clinical chemistry, clinical microbiology, and hematology), or in pathologic anatomy (for qualification in neuropathology)—two additional years of supervised training in the special field of their choice in institutions approved by the Council on Medical Education and Hospitals of the American Medical Association or by the Board.
2. Applicants not holding a certificate in pathologic anatomy or clinical pathology—five years of training in the special field of their choice, provided four of the five years have been in institutions approved by the Council on Medical Education and Hospitals of the American Medical Association or by the Board. Candidates may, at their own election, substitute not to exceed 12 months of a straight or rotating clinical internship or a fellowship or instructorship in any of the preclinical departments of a university for one of the four years. The fifth year may be a continuation of supervised training or may be independent practice of the particular specialty in a hospital approved by the American Medical Association or in other institutions acceptable to the Board.

D. Special Qualifications—Certification Without Examination

Prior to January 1, 1960, the Board at its discretion may certify candidates without examination if the following conditions have been met as of Jan. 1, 1955:

1. That the candidate has been for a period of five years of professorial rank in the special field of his choice and in an approved medical school, or
2. That the candidate has been practicing his specialty for 10 years in a senior position in a hospital having an adequate department in the special field, approved by the Council on Medical Education and Hospitals of the American Medical Association, or in an institution acceptable to the Board.

Grants for Scholars in Cancer Research.—Applications for grants for Scholars in Cancer Research are being accepted by the Committee on Growth of the National Research Council, acting for the American Cancer Society.

These awards are designed to bridge the gap between the completion of fellowship training and the period when the young scientist has thoroughly demonstrated his competence as an independent investigator. A grant of $18,000, payable over three years, will be made to each scholar's institution as a contribution toward his support, his research, or both. Each institution may submit more than one application. These grants are not restricted to the support of persons who have held American Cancer Society fellowships. Applications should be submitted by institutions on behalf of a candidate prior to Jan. 1, 1955.

Application blanks and additional information may be obtained from the Executive Secretary, Committee on Growth, National Research Council, 2101 Constitution Avenue, N. W., Washington 25, D. C.

Funds Needed to Combat Antivivisectionists.—Calling attention to the fact that "anti-vivisectionists are preparing for all-out attempts to restrict animal experimentation in several key areas," Anton J. Carlson, Ph.D., president, National Society for Medical Research, appeals for funds to put into effect the society's plans to meet this challenge. Contributions may be sent to the society's headquarters, 208 N. Wells St., Chicago 6.

Cancer Research Supplement.—In April, 1953, a supplement to Volume 13 of *Cancer Research* was published to present a backlog of negative data on compounds tested in experimental cancer chemotherapy programs. A second supplement, made possible by a grant from the National Cancer Institute of the United States Public Health Service, is planned for publication early in 1955. This will present additional negative data, and also data from clinical cancer chemotherapy programs. Material for inclusion in the supplement is invited; it must be received by one of the editors before Oct. 15. For information regarding preparation of data for submission, communicate with C. Chester Stock, Sloan-Kettering Institute for Cancer Research, 410 E. 68 St., New York 21.

Research Policy of Tobacco Industry Research Committee.—New York, July 27, 1954, Dr. Clarence Cook Little, Scientific Director of the Tobacco Industry Research Committee, announced the three main areas to be covered by the research program being financed by the tobacco industry group.

In order to find conclusive facts concerning questions that have been raised about tobacco use and health, Dr. Little said, the Scientific Advisory Board of the Tobacco Industry Research Committee has adopted a three-fold policy which will direct funds into research on the following: (1) Study of the physical and chemical composition of tobacco and accompanying products. (2) Study of tissue changes in humans and in animals under various conditions. (3) Study of smoking and other tobacco habits and of the emotional and physical make-up of smokers.

The Scientific Advisory Board, a group of outstanding scientists guiding the Committee's research activities, is completing its review of the first applications for specific research projects.

The Tobacco Industry Research Committee was set up early this year by leading cigarette manufacturers, organizations of growers, and tobacco warehouse associations. Its purpose is to finance objective research on tobacco and health. Grants will be made to qualified individuals and institutions who will report their own findings. Dr. Little pointed out that the industry has made available an initial fund of $500,000 to be augmented as the need is developed by recommendations of its Scientific Advisory Board.

Died.—Augustus B. Wadsworth, past president of the Association of Pathologists and Bacteriologists, and retired director of laboratories and research for the New York State Department of Health, New York, died on June 1, 1954, aged 81.

New Chairman of Pathology Department.—Dr. Joseph E. Flynn, associate professor of pathology at the Columbia University College of Physicians and Surgeons, New York, has been appointed professor of pathology at the University of Missouri School of Medicine, Columbia, effective July 1. He replaces Dr. M. Pinson Neal who will continue as professor of pathology.

Elections of American Society for Experimental Pathology.—At the annual business meeting of the American Society for Experimental Pathology held at Atlantic City, N. J., April 14, 1954, the following officers were elected for the year July 1, 1954 through June 30, 1955:

President	Russell L. Holman
Vice President	Harold L. Stewart
Secretary-Treasurer	Cyrus C. Erickson
Councilors	Frank W. Hartman
	Emory D. Warner

The next annual meeting is to be held in San Francisco, April 10-16, 1955.

Doctor Draft.—The National Advisory Committee to Selective Service has warned that unless young physicians deferred for the last year to complete internships or residencies apply for commissions the government will have to start calling up priority 3 doctors over 31 years of age. In a report to local draft boards, the committee recalled that as early as last May it had advised doctors in priorities 1 and 2 and those in priority 3 under age 31 who were slated to finish their internships and residencies this July to apply at once for commissions. In that way, the May notice explained, there would not be a protracted period of waiting between the end of the hospital year and the call to active duty.

This warning apparently went unheeded by many doctors. On July 7 the committee suggested to draft boards that they recheck their draft files of young doctors given educational deferments and that they terminate deferments if they had not applied for commissions. The committee commented, "These are the groups that are most urgently needed to meet the future calls during the present fiscal year." It added, "The committee is deeply concerned over the possibility of calling up physicians in priority 3 over 31 years of age to meet military needs for fiscal 1955. The purpose of the procedure outlined is to assist in avoiding that possibility since many of the older group are in well-established practices and are more essential to hospitals and medical schools."

International Symposium.—An international symposium on "Problems in Physiology and Pathology of the Eye" will be held at the State University of Iowa College of Medicine, Sept. 24-25. The program will include: Gunnar von Bahr, Uppsala, Sweden, "Corneal Thickness: Its Measurement and Changes"; Giambattista Bietti, Parma, Italy, "Ophthalmic Problems in Diabetes and Hypoglycemia"; Hans Goldmann, Berne, Switzerland, "Slit-Lamp Microscopy of the Fundus"; Eugene Wolff, London, "Ophthalmic Pathology of Fat"; G. B. J. Keiner, Zwolle, Netherlands, "Optomotor Reflexes"; H. K. Müller, Bonn, Germany, "Phosphate Metabolism of the Lens"; J. Boeck, Gras, Austria, "Periarteritis Nodosa and Related Diseases"; Harold Henkes, Rotterdam, Netherlands, "Electroretinography."

New York Academy of Medicine Annual Graduate Fortnight.—Infections and their management will be the subject of the 27th annual graduate fortnight of the New York Academy of Medicine, to be held Oct. 18-29. The program will include 21 evening lectures, 6 morning panel meetings, 10 hospital clinics, and a scientific exhibit. For programs and other information, address the Secretary, Graduate Fortnight, 2 E. 103 St., New York 29. Nonfellows of the Academy may register.

Clinical Pathologists to Meet in Washington.—The International Congress of Clinical Pathology will be held in Washington, D. C., Sept. 6 to 11, under the sponsorship of the International Society of Clinical Pathology and on the invitation of the American Society of Clinical Pathologists. The joint opening session will be Monday morning. At 2 p. m. Dr. Emma S. Moss, New Orleans, will serve as moderator for a symposium of the College of American Pathologists on diseases caused by fungi. Tuesday morning a joint scientific session on the geographical distribution of cancer will be opened by "General Incidence of Cancer Throughout the World" by Harold F. Dorn, Ph.D., Bethesda, Md. Dr. Paul E. Steiner, Chicago, will contribute a paper entitled "Etiologic Significance of Racial Studies in Cancer." A joint scientific session on geographical distribution of diseases other than cancer will be held Friday afternoon, at which time the Askanazy lecture of the International Society of Geographic Pathology will be delivered. Saturday morning will be devoted to the annual seminar of the American Society

of Clinical Pathlogists. Presidents of the participating groups are Dr. John R. Schenken, Omaha, American Society of Clinical Pathologists; Dr. R. Kourilsky, Paris, France, International Association of Clinical Pathology; Dr. Harold Stewart, Bethesda, Md., International Association of Medical Museums, and Dr. Robert A. Moore, Pittsburgh, International Society of Geographic Pathology.

Clinical Fellowships of the American Cancer Society.—A limited number of fellowships offer graduates in medicine opportunities for postgraduate training, emphasizing diagnosis and treatment of cancer.

Fellowships available on and after July 1, 1955 will be awarded for one year and are renewable to and including three years.

Fellowships are awarded to *institutions* only upon application by deans, executive officers, or department heads. Individuals desiring such Fellowships should consult the appropriate authority in the institution of their choice.

Applications for Fellowships for the year 1955-1956 must be submitted prior to Sept. 15, 1954. Further information may be obtained from American Cancer Society, Professional Education Section, 47 Beaver Street, New York 4.

Citations.—Dr. Dorothy H. Andersen, Assistant Professor of Pathology, Columbia University College of Physicians and Surgeons, has been given one of the Elizabeth Blackwell citations of the New York Infirmary, in recognition of her work in relation to cystic fibrosis of the pancreas.

Status of Cancer Knowledge Is Suiter Lecture Topic.—Six authorities on cancer will participate in the 1954 A. Walter Suiter Lecture, Nov. 4, at the New York Academy of Medicine, in the form of a symposium entitled Cancer: What We Know Today.

The program, which is planned particularly to give the general practitioner a well-rounded view of the present status of knowledge about cancer, has four parts. The first three topics and the speakers will be:

Changing Incidence of Cancer Over the Years. Harold F. Dorn, Ph.D., Chief, Office of Biometry, National Institutes of Health, Bethesda, Md.

Multiple Views on the Causation of Cancer. Dr. Harold L. Stewart, Chief, Laboratory of Pathology, National Cancer Institute, Bethesda, Md.

The Natural History and Diagnosis of Cancer. Dr. Lauren V. Ackerman, Professor of Surgical Pathology and Pathology, Washington University School of Medicine, St. Louis.

The fourth topic will be Modern Therapeutic Measures in Cancer and Their Effectiveness, with the following speakers:

Surgery. Dr. Owen H. Wangensteen, Professor of Surgery, University of Minnesota Medical School, Minneapolis.

Radiology. Dr. Richard H. Chamberlain, Associate Professor of Radiology, University of Pennsylvania School of Medicine, Philadelphia.

Chemotherapy. Dr. Alfred Gellhorn, Director, Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York.

The Suiter Lecture is presented under the auspices of the Academy's Committee on Public Health Relations, to which Dr. Suiter at his death, in 1925, left a legacy to finance lectures in public health or legal medicine, his two principal personal interests. For this symposium the New York City Cancer Committee is cooperating with the Committee on Public Health Relations.

Dr. Suiter, a native of Herkimer, N. Y., graduated from Columbia University College of Physicians and Surgeons in 1871 and spent his life practicing in Herkimer. He was greatly concerned with medical jurisprudence, having served as an expert in both civil and criminal cases. He was among those instrumental in the establishment of the state board of medical examiners and served as a member of the board for several years. In public health he took particular interest in problems of municipal sanitation.

Second Annual Antibiotic Symposium.—The Second Annual Antibiotic Symposium under the supervision of the Department of Health, Education and Welfare will be held on Oct. 25, 26, 27, and 28, 1954, in the Department of Health, Education, and Welfare auditorium, 4th Street and Independence Avenue, S. W., Washington, D. C. The last date for submission of titles and abstracts is Sept. 15.

Appointments.—Dr. Bernard M. Wagner, research assistant in the department of pathology, Mount Sinai Hospital, has been made assistant professor of pathology at the Hahnemann Medical College and Hospital of Philadelphia. In his new position he is to be in charge of experimental pathology.

Pasteur Award.—The Society of Illinois Bacteriologists has given the Pasteur Award for 1954 to James A. Reyniers, M.S., research professor of bacteriology, University of Notre Dame, "for giving to science a new and powerful tool, the germ-free animal technique." Dr. Reyniers and his associates have developed techniques which have made it possible for animals to be born, live a normal life span, and die, without ever coming into contact with germs.

McIntyre-Saranac Conference.—A conference on silicosis and occupational chest diseases jointly sponsored by the McIntyre Research Foundation of Toronto, Canada, and the Saranac Laboratory of Saranac Lake, New York, has been arranged for Monday, Tuesday, and Wednesday, Feb. 7, 8, and 9, 1955, in the Town Hall at Saranac Lake.

Anthony J. Lanza, M.D., formerly director of the Institute of Industrial Medicine and now emeritus professor of industrial medicine at New York University-Bellevue Medical Center, has been named chairman of the conference. He is also chairman of the scientific program committee which comprises Dudley Irwin, M.D., Medical Director, Aluminum Company of America, Pittsburgh; Guy Hannon, M.D., Medical Director, McIntyre Research Foundation, Washington, Pa.; Gordon M. Meade, M.D., Executive Director of Trudeau-Saranac Institute; and Gerrit W. H. Schepers, M.D., Director of the Saranac Laboratory.

The business arrangements including reservations will be handled by Norman R. Sturgis Jr., and the treasurer will be Clarence L. Wagner, both of the Trudeau-Saranac Institute staff. All communications concerning the conference should be addressed to Mr. Sturgis, Saranac Laboratory, Saranac Lake, New York.

Books

Fundamentals of Otolaryngology: A Textbook of Ear, Nose and Throat Diseases. By Lawrence R. Boies, M.D. Second Edition. Price, $7.00. Pp. 907 with 304 illustrations, 7 in color. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; W. B. Saunders Company, Ltd., 7 Grape St., Shaftesbury Ave., London W.C. 2., 1954.

This is the second edition of a book that has been used as a standard text in many medical schools. The general organization of the first edition is retained. "An Introduction to Modern Otolaryngology" has been added before the first chapter with a timely evaluation of the current status on trends in the specialty. A new chapter on "Bronchoscopy and Bronchography in Pulmonary Diseases" briefly reviews recent advances. There is a new addendum on "The Possibilities of Transudate Disorders in Otolaryngology: Allergy, Anatomic Dysfunction and Endocrine Imbalance."

Three chapters on middle ear disease are reorganized with a broader consideration than otitis media. The general chapter on therapy, as well as the specific therapy throughout the text, has been brought up to date and more consideration is given to antibiotics, antihistamines, and corticotropin (ACTH) and cortisone.

The format remains the same, but the illustrations have been moved to the lower part of the page, resulting in easier-reading text.

The second edition retains the same characteristics that made the first popular, but it has been expanded, with some reorganization, and has been brought up to date.

Pathology for Students of Dentistry. By George L. Montgomery, T.D., M.D., Ph.D., F.R.F.P.S.(G.), F.R.S.E.; St. Mungo (Notman) Professor of Pathology, University of Glasgow. Price, $7.50. Pp. 305, with 133 figures. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2 (E. & S. Livingstone, 16 and 17 Teviot Place, Edinburgh 1), 1953.

Those associated with the teaching of general medical subjects to dental students have long felt the need for such books as this. Much of the difficulty of determining the proper exposure of dental students to general and systemic pathology stems from the lack of a textbook written with insight into their interests and needs.

The arrangement of the subject matter is of some interest. The book commences with a discussion of Local Reactions to Trauma and ends with General Reactions to Trauma. The description of the primary repair process precedes the treatment of the acute inflammatory reaction, and there are other departures from conventional patterns. However, these are lucid and acceptable. Systemic pathology is represented by sections dealing adequately with the circulatory and respiratory systems, with peptic ulceration (surely of importance to the hard-working dentist), and with the lymph nodes. It is unfortunate and not clearly understandable that diseases of the liver and kidneys were omitted entirely, and one experiences a certain nostalgia on failing to find a section of pigment deposition. Of the three hundred pages in the book, ninety-one are devoted to inflammation and infection, and eighty-one to neoplasms.

Mastopathie und Milchdrüsenkrebs, Zugleich zweite Auflage von Pathologie, Klinik und Behandlung der Mastopathie. By Prof. Dr. Georg E. Konjetzny. DM 25. Pp. 7 + 140, with 48 pages of illustrations. Ferdinand Enke, Hasenbergsteige 3, Stuttgart, W, 1954.

This concise monograph, dealing with the various forms of chronic cystic mastitis and its relation to mammary cancer, is a revision and enlargement of an earlier book. It includes dis-

cussions on the pathology, clinical manifestations, course, and management of the disease, as well as an extensive bibliography. The author is convinced of a relationship to cancer by his experiences. These discussions are illustrated by good photomicrographs, which appear to have been interpreted very liberally.

Aids to Pathology. By John O. Oliver, M.B., B.S.(Lond.), M.R.C.S.(Eng.), L.R.C.P.(Lond.); Director of Pathology, St. John's Hospital for Diseases of the Skin, London; Lecturer to the Institute of Dermatology; Examiner in Pathology to the Examining Board in England; Late Director of Pathology, Southend-on-Sea Hospitals Management Committee; formerly Lecturer in Pathology and Bacteriology and Assistant Pathologist, St. Thomas's Hospital, London, and Pathologist and Bacteriologist, Royal Eye Hospital and General Lying-In Hospital, London. Tenth Edition. Price, $2.50. Pp. 343, with 16 figures. Baillière, Tindall & Cox, 7-8 Henrietta St., Covent Garden, London, W.C.2, 1953.

The first edition of this book was published in 1907, and the present (tenth) edition retains the fluent and somewhat verbose style of that period. This is rather unfortunate, since the volume is intended to be a condensation of the material presented in a course in anatomic pathology. As a result, it seems that explanations and explorations which would help the student to gain understanding, rather than historical quotations, adjectives, and descriptions which could be replaced by illustrations, are sacrificed to brevity. The few illustrations included are rather primitive line drawings.

New material and revisions have been incorporated in the sections on nephritis, hepatitis, infection, and others, but lower nephron nephrosis is neglected.

This is essentially an old-fashioned book to help students cram for old-fashioned examinations. It is not likely that it will replace Anderson's "Synopsis of Pathology" in the United States.

Surgical Forum: Proceedings of the Forum Sessions, Thirty-Ninth Clinical Congress of the American College of Surgeons, Chicago, October, 1953. By Surgical Forum Committee, I. S. Ravdin, M.D., Chairman. Price, $10.00. Pp. 752, with 131 figures. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; W. B. Saunders Company, Ltd., 7 Grape St., London, W.C. 2, 1954.

This volume contains ten introductory essays and one hundred thirty-eight original papers constituting reports of experimental work presented at the Surgical Forum Sessions of the October, 1953, meeting of the American College of Surgeons. The papers are arranged under the ten broad headings of heart and great vessels; blood vessels and circulation; lungs; esophagus, stomach, and intestine; liver and pancreas; burns and renal function; shock, nutrition, and electrolytes; wounds, infections and antibiotics, and anesthesia; cancer and steroids, and plasma expanders. Each article is a self-contained brief account of the problem concerned, method of study, and experimental results. In addition, there is usually a discussion of the significance of the findings and a list of pertinent references. Naturally, each presentation is concerned with a limited and specific problem, but the range covered by the entire volume is immense, both as to subject matter and as to techniques of study. Each paper is carefully prepared and authoritative, and the collection is valuable because it contains a large amount of recent original work.

These Surgical Forum volumes should be of great interest to all in surgery and related fields, as they bring together new contributions that would otherwise appear in diverse journals. This particular issue is of high caliber, is nicely printed and edited, and can be recommended with enthusiasm.

Tissue Culture. By E. N. Willmer, M.D. Second Edition. Price, $2.25. Pp. 175, with 11 illustrations. John Wiley & Sons, Inc., 440 4th Ave., New York 16, 1954.

This little monograph, one of Mathews Monographs on Biological Subjects, first appeared in 1935 and is now revised to take into account the developments in ideas and techniques of the intervening years. In the first half of the book the author describes tissue culture methods, characteristics of growth of cells in vitro, the culture medium, and the metabolism of growing cells. In the second part he describes some of the characteristics of organized growth and differentiation in tissue culture. The book has a brief but useful glossary of terms used by the experts. It has a fresh and extensive bibliography.

The most interesting and, to this reviewer, important quality of this little book is that it describes not only technical details but also certain important biologic processes. The author clearly recognizes that tissue culture is but one way, albeit an important modern way, of examining biologic processes. He describes what one sees with this method and discusses what the importance of this way of observing may be in biologic investigations. This makes the book a useful and worth-while acquisition, not only for those who might intend seriously to do tissue culture work but also for those who are interested in what role tissue culture plays in modern biologic and medical investigation. On the one hand, one can use the information in the book as a background for critically evaluating published experiments, and on the other hand, one can read the small volume as an excursion in modern experimental biology. It is, on the whole, well written. It is clearly evident from the above that the reviewer highly recommends this monograph to anyone interested in the mechanisms of cell biology, be he experimenter or practitioner.

Verhandlungen der deutschen Gesellschaft für Pathologie. Edited by E. Randerath. Pp. 396, with 193 illustrations, tables and charts. Gustav Fischer, Villengang 2, Jena 15b, Germany, 1954.

The Size and Growth of Tissue Cells: Publication No. 172, American Lecture Series; Monograph in American Lectures in Medical Physics. By Joseph G. Hoffman, Ph.D., Director of Cancer Research, Roswell Park Memorial Institute; Research Professor of Biophysics, School of Medicine, University of Buffalo; Consultant, Los Alamos Scientific Laboratory, University of California, Los Alamos, N. Mex. Edited by Otto Glasser. Price, $4.00. Pp. 128, with 13 illustrations. Charles C Thomas, Publisher, 301-327 East Lawrence Avenue, Springfield, Ill., 1953.

When, for the most sophisticated work with the electron, ultraviolet, or infrared microscope, cells are consciously selected for measurement, a number of implicit assumptions come into play, derived from the size, shape, and appearance of these cells in the light microscope, which reflect a judgment as to the normality or abnormality of these cells. The author of this interesting volume traces the history of such concepts of cells from the pioneer studies of Lebert in 1851. This was only 12 years after the promulgation of the cell theory itself, and at a time before the doctrine that cells may only arise from other cells was well accepted. Since that time, cells, nuclei, and nucleoli have been frequently measured, with more or less precision, or their sizes have been visually assessed, and while the issue has been bitterly debated, the idea that the size and proportions of cancer cells differ from normal cells has persisted. The author emphasizes the importance of the frequency distribution of cell sizes as a primary parameter in determining their growth characteristics. While much of the material is of great intrinsic interest to cell physiologists and pathologists alike, the volume is not always lucid reading. Heidenhain's Law, for example, is mentioned several times in important contexts, but is never explicitly defined, nor is the primary reference given. Perhaps not all readers are so conversant with Kelvin's tetradecakaihedron as to be able to visualize easily the geometry of a plane section of it; a line drawing would have elucidated it completely. Also, though the author leaves the question open, the problem of cell fixation without shrinkage would seem to have been essentially solved by the Altman-Gersh freeze-drying technique. The volume is recommended for its fresh viewpoint on old and current problems in cell physiology.

Symposium on Protein Metabolism: Proceedings of the Nutrition Symposium Held at the University of Toronto, Toronto, Canada, Oct. 30, 1953. Price, $1.50. Pp. 107. The National Vitamin Foundation, Inc., 15 East 58th Street, New York 22, 1954.

Newer Concepts of the Causes and Treatment of Diabetes Mellitus: Proceedings of the Symposium on Diabetes Sponsored by the New York Diabetes Association and Held at Memorial Hospital and The New York Academy of Sciences, New York, Oct. 8, 1953. Price, $2.50. Pp. 181. The National Vitamin Foundation, Inc., 15 East 58th Street, New York 22, 1954.

Altern und Krankheit: Grundlagen einer biorheutischen Nosologie. By Prof. Dr. Med. Max Bürger, Direktor der Medizinschen Universitätsklinik, Leipzig. Second Edition. Pp. 600, with 285 figures and 223 tables. Price, DM 40. Georg Thieme; Thomaskirchof 21 (10b), Leipzig C1; agents for the U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1954.

Klinische Darmbakteriologie für die Ärztliche Praxis. By Prof. Dr. Med. et Phil. T. Baumgärtel Price, $3.40. Pp. 132. Georg Thieme, Diemershaldenstrasse 47 (14a), Stuttgart-O; agents for U. S. A.: Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1954.

Nature and Structure of Collagen: Papers Presented for a Discussion Convened by the Colloid and Biophysics Committee of the Faraday Society at King's College, London, on 26 and 27 March, 1953. Edited by J. T. Randall, F.R.S., Honorary Director, Medical Research Council Biophysics Research Unit, and Wheatstone Professor of Physics, University of London. Price, $6.50. Pp. 269, with numerous illustrations. Academic Press Inc., 125 E. 23rd St., New York 10, 1953.

This symposium brings together from British sources, subject matter which has occupied several conferences in the United States under the auspices of the Josiah Macy Jr. and the Eli Lilly Foundations. As befits a Faraday Society meeting, emphasis is on physical and biophysical aspects. Topics in this realm include the ultrastructure of collagen, be it derived from bone, cartilage, teeth, vertebral disc, or from the attachment threads of the oyster, studied with the electron microscope, with x-ray diffraction, and with the refractometer, at a high level of technical achievement. Biochemical studies of gelatin and collagen are reported by several authors. At a level where physics, chemistry, and biology meet, there is an excellent review of fibrogenesis by Miss Fitton Jackson. This author and Randall also discuss the problem of collagen cross-striation in a particularly illuminating manner. On the biological side, the contributions are uneven, and a certain confusion is compounded by the scattering of topics at random throughout the book. It would seem that the second article, on collagenase, were better deferred till later, when the various enzyme substrates of connective tissue had been discussed. The purely histological parts of the symposium are disappointing. On the other hand there is a good appreciation, and, surprisingly, at the biochemical level, of the interaction of the fibrillar elements and an amorphous, mucoprotein-containing extracellular and interfibrillar material, the ground substance. In this regard, the finding of Consden that the bulk of this material, in the subcutaneous connective tissue, is uronic acid free, seems a most significant advance. This volume is warmly recommended for the frequent demonstration of the use of precise measurement plus imagination in the interpretation of that fascinating substance, the connective tissue.

An Introduction to Bacterial Physiology. By Evelyn L. Oginsky and Wayne W. Umbreit. Price, text, $6.00, trade, $7.25. Pp. 416, with 94 figures, 6 drawings, and 18 tables. W. H. Freeman & Company, 549 Market St., San Francisco 5, 1954.

The rapid progress and increased interest in recent years in the field of bacterial physiology have led to a number of texts or reference books devoted either largely or entirely to this subject. The book by Oginsky and Umbreit is the latest of some seven or eight books, either new contributions or revised editions of older works, which have appeared since 1945.

The book is divided into six sections. The first is a brief discussion of the nature of bacterial physiology. The second section is devoted to cytology and cytochemistry of the bacterial cell. The third discusses bacterial populations, with chapters on growth, nutrition, chemical and physical environment, and genetics. The fourth, on metabolism, treats of enzymes, energy, dehydrogenation and respiration, metabolism of carbohydrates, of other substances, and amino acids and proteins. Section five is devoted to the "self-reliants" and "dependents," the autotrophic and photosynthetic bacteria, the viruses and rickettsia. The sixth deals with capacities of the bacterial cell, adaptation, survival, and virulence as a physiological problem.

The authors state they purposely set the level of this book to bridge the gap between general bacteriology and more advanced physiological information. This objective calls for selectivity in choice of material rather than completeness or emphasis upon the latest information. In general, the aim has been accomplished. Although there is some duplication of material included in general bacteriology texts, the repetition for the most part serves the purpose of introducing the discussion of more advanced material. An obvious effort has been made to keep the discussion brief and to the point. Frequently, when presenting controversial material, different current ideas are stated in brief summaries. The style is clear and readable; generous use is made of well-selected charts and figures, some of which depart from the customary type found in most bacteriology texts and portray interesting imaginary representations of events, as for example, a particle approaching a bacterial cell. Each chapter contains a list of selected references and some questions about the material. Although some of the discussions are rather brief, nevertheless the book should well serve its purpose as an introduction to bacterial physiology.

1954 Current Therapy. Edited by Howard F. Conn, M.D. Price, $11.00. Pp. 898. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; W. B. Saunders Company, Ltd., 7 Grape St., Shaftesbury Ave., London, W. C. 2, 1954.

This is a tremendous volume containing methods of treatment for practically every disease state. Its real worth lies in the details allowed the numerous authors. Time tables, diet lists, and day to day dosage forms with minute variations are given. Some of the articles are remarkable in their completeness although only a few are long, and these seem to be the more complicated and grouped disorders such as tuberculosis. In a considerable number of instances more than one author outlines the treatment and all are honestly titled "method of Dr.——." There is occasional brutal honesty when it is said that there is no treatment. A number of the presentations are outstanding such as "pulmonary tuberculosis," "cardiac arrhythmias," "the purpuric states," "peptic ulcer," and "trauma of the nervous system."

This book is intended as a reference manual for the busy physician, and as such it ably accomplishes this purpose. It is a good book also for pathologists to have at hand, in view of the pathologic consequences of therapeutic procedures seen not infrequently in surgical pathology and at necropsy.

Histology. Edited by Roy O. Greep, Ph.D., Dean and Professor of Dental Science, Harvard School of Dental Medicine, with thirteen contributors. Price, $15.00. Pp. 953, with 648 illustrations. The Blakiston Company, 575 Madison Ave., New York 22, 1954.

Originally intended as a new edition of "A Textbook of Histology" first written by Bremer and Weatherford, this revision by the microscopic anatomy group at the Harvard medical and dental schools proved so extensive that it was decided to issue it as a new textbook. The result has been a readable, fundamental book of histology which, while emphasizing descriptive anatomy, interrelates structure and function in order to give a complete appreciation of the total organism. Histochemical techniques have been given an encouraging amount of space in these correlating descriptions, though at times such findings are presented in a manner which may cause confusion in the minds of beginning students.

As with any book with multiple contributors, there is considerable variation in the style and lucidity of writing from chapter to chapter. Also, some chapters, notably those on blood and blood formation and on the vascular system, were apparently written earlier and fail to include some of the more recent work in their respective fields. The volume is profusely illustrated, many illustrations being schematic drawings or camera lucida reproductions. A few illustrations are in color. The quality of illustrations is adequate, though some reproductions are not as distinct as would be desirable.

As a textbook of general histology the present volume is satisfactory. The goal of integration of form with function is admirable, and the recognition of the value of histochemical techniques for such integration is gratifying. The methods of presentation and styles of writing are not uniform and at times may prove confusing for the beginning student.

Cytoarchitecture of the Human Brain Stem. By Jerzy Olszewski, M.D., Ph.D. and Donald Baxter, M.D. Price $16.00. Pp. 199, with 185 plates. J. B. Lippincott Company, 227-231 S. 6th St., Philadelphia 5; Aldine House, 10-16 Bedford St., London, W.C. 2, 1954.

The suggestion of the Vogts several years ago of the need for a detailed investigation of the reticular formation of the brain stem served as the stimulus for the present volume. Knowledge of both morphological and functional organization of the reticular formation was necessary for and understanding of extrapyramidal diseases. Accordingly, a study of normal cytoarchitecture was undertaken by the authors. This atlas is the result of that study.

The atlas shows not only the location of the various brain stem nuclei but also notes the peculiar groupings of cells and special characteristics of individual cells. Multiple representative cross sections through a brain stem were selected. These sections were photographed, enlarged 60 diameters, and the boundaries of nuclei outlined on the photographs. The photographs then were reduced to 15 diameters for publication. Such a method provides semischematic drawings that are accurate representations of the relative size, orientation, and density of neurones in different areas. These drawings are augmented by photomicrographs at both low and high magnifications and by descriptions of anatomical features of individual nuclei.

The monograph is an excellent addition to the anatomical literature of the human brain. It presents in detailed analyses the various nuclei of the brain stem and brings together in one volume the present knowledge of the anatomy of this region. The text is clear, the illustrations, in black and white, are numerous and distinct. This atlas will prove invaluable to those persons concerned with the anatomy and pathology of the human brain stem.

A Curriculum for Schools of Medical Technology. Edited by Israel Davidsohn, M.D., Chairman of Department of Pathology, Chicago Medical School, and Kurt Stern, M.D., Assistant Professor of Pathology, Chicago Medical School, and five other contributors. Third Edition. Price, $3.00. Pp. 122, with no illustrations. Registry of Medical Immunologists of the American Society of Clinical Pathologists, P. O. Box 1209, Muncie, Ind., 1954.

The "Curriculum" continues to spearhead the efforts of the Council on Medical Education and Hospitals of the American Medical Association and the Registry of Medical Technologists of the American Society of Clinical Pathologists in maintaining high and uniform standards for the training of medical technologists.

Since its first edition, published in 1937, this volume has attempted to direct educators in planning a curriculum for medical technologists which would insure well-rounded and modern knowledge and understanding of clinical laboratory procedures.

The suggested curriculum seems well-planned and well-balanced but consideration might be given in future editions to recommending that the period of supervised practical experience in histotechnique be extended at a slower pace over a longer period of time while the students are studying other subjects more intensively. This subjective branch of medical technology, more than most others, requires a carefully trained eye and manual skill which is difficult for many students to master in four to six weeks. It is likely that such a step would, in time, aid materially in improving the quality of hospital histological preparations the country over.

The third edition brings up to date the references and lists of skills which should be taught. The low price of the manual, which is not published for profit, should facilitate its purchase by students of medical technology and all those who train technologists.

The Biochemistry of Clinical Medicine. By William S. Hoffman, M.D., University of Illinois. Price, $12.00. Pp. 681, with 58 figures and 50 tables. Year Book Publishers, Inc., 200 East Illinois St., Chicago 11, 1954.

This book is an attempt to present modern biochemical findings that will be of aid to the clinician. As stated by the author in the preface, the book is aimed at the level of the general practitioner and is thorough rather than elementary. Recent graduates may be disappointed in the proportion of space given biochemistry in comparison to clinical discussion.

The selection of material and its organization are somewhat unusual. This is based on the author's personal experience and interest. Complete clinical essays are presented on diabetes

mellitus, Bright's disease, hepatitis, cirrhosis and gout. The book is written in accepted medical style and there are a few well chosen illustrations. It is well indexed and provides a single volume readily available source book on the level stated by the author.

Muscular Contraction. By M. Dubuisson, Ph.D. Price, $6.50. Pp. 243, with 34 figures. Charles C Thomas, Publisher, 301-327 East Lawrence Ave., Springfield, Ill., 1954.

This monograph from the American Lectures in Biochemistry and Biophysics is a detailed and orderly review of the problems of muscular contraction. It consists of three parts, a well-documented account of the chemical composition of the muscle cell, a review of the location of the different constituents in the muscle fiber, and the physicochemical aspects of muscular contraction. The author's aim, as stated in the preface, is "to assemble not all, but some of the important information on these subjects: to try to find out where we are, not to formulate a theory concerning muscular contraction." This book will be of interest mainly to those working actively in this field.

The Microtomist's Formulary and Guide. By Peter Gray, Ph.D., Head, Department of Biological Science, University of Pittsburgh. Price, $10.50. Pp. 794, with 87 illustrations. The Blakiston Company, 575 Madison Ave., New York 22, 1954.

Here is a difficult job well done. Every so often the accumulated experience and knowledge on a subject must be brought together, classified, and made useful for those who are going to extend it to the next milestone.

Professor Gray has recorded in one book a wealth of practical information which a beginning technician will find useful, easy to understand, and authoritative, combined with a complete classified formulary and bibliography of all the world's literature on fixatives, stains, embedding media, mounting media, etc. He even includes formulas for high-quality label adhesives so that the microscopist's labels will not come off the slide.

While no one laboratory will probably ever use all of the information in this book, it should be well worth its purchase price to any laboratory because of the many hours it will save in tracking down seldom-used procedures and in avoiding the mistakes of others.

Every writer of an article on staining should acquire wisdom in the witty introduction. Everyone should enjoy the helpful drawings which illustrate the principles and pitfalls of microtechnique.

The Meaning of Social Medicine. By Iago Galdston, M.D. Price, $2.75. Pp. 137, with no illustrations. Harvard University Press, Cambridge 38, Mass., 1954.

This small volume deals with one of the most important problems in medical education today. What is the over-all aim of medicine and consequently of medical education? Dr. Galdston attempts to show that the current philosophy in medical practice and education is based upon the teaching and investigations done primarily in the latter half of the nineteenth century. At this time, the concepts of simple and specific cause and specific therapy of disease processes were developed and became firmly entrenched. Dr. Galdston admits that this philosophy and the work which it engendered have great merit, but he believes that a broader outlook is necessary in medicine. It is his contention that the point of view in medicine should be shifted from the morbid process and death to a broader biological basis. He argues that attention should be focused upon health, life, and the full development of life's potentialities. It is in this light that he reviews the social possibilities of medicine and medical care. Social medicine, as he points out, is not merely socialized medicine. It is not merely, if at all, governmental provision of standard medical care, public health, and preventive medicine. Social medicine is a philosophy concerned with the nature of medicine and its relationship to society.

This is a very readable and a thought-provoking little book, and well worth the price and the time involved in acquiring its substance. Dr. Galdston is Executive Secretary to the Committee on Medical Information, The New York Academy of Medicine; Lecturer in Public Health, New York Medical College, Flower and Fifth Avenues Hospital, and Chairman, Committee on Inter-

national Relations, American Psychiatric Association. This breadth of interest and experience lends weight to Dr. Galdston's thoughts. Any "student" of medicine, will be most interested in reading this book.

Endemic Goiter: The Adaptation of Man to Iodine Deficiency. By John B. Stanbury, M.D.; Gordon L. Brownell, Ph.D.; Douglas S. Riggs, M.D.; Hector Perinetti, M.D.; Juan Itoiz, Ph.D., and Enrique B. Del Castillo, M.D. Price, $4.00. Pp. 209. Harvard University Press, Cambridge 38, Mass., 1954.

This book describes the results of an adventure in modern biology. It is unusual today that the opportunity arises for the study of the endemic iodine deficiency state with the currently available concepts and techniques. In this book is described the results of the joint venture between a group of American and Argentinian clinicians. These investigators were able to examine 129 people in Mendoza, Argentina, an area long-known to be a goiter region. In a nicely designed series of experiments, they made use of iodine isotope techniques to study the metabolism of this substance under the conditions of a state of chronic deficiency. The data thus collected were subjected to statistical and mathematical analysis of an elegant type still infrequently seen in modern medical studies. The results and the analyses of these studies are of considerable interest, not only to those specifically involved in the field of thyroid physiology and iodine metabolism, but also to anyone interested broadly in the fields of endocrinology and metabolism.

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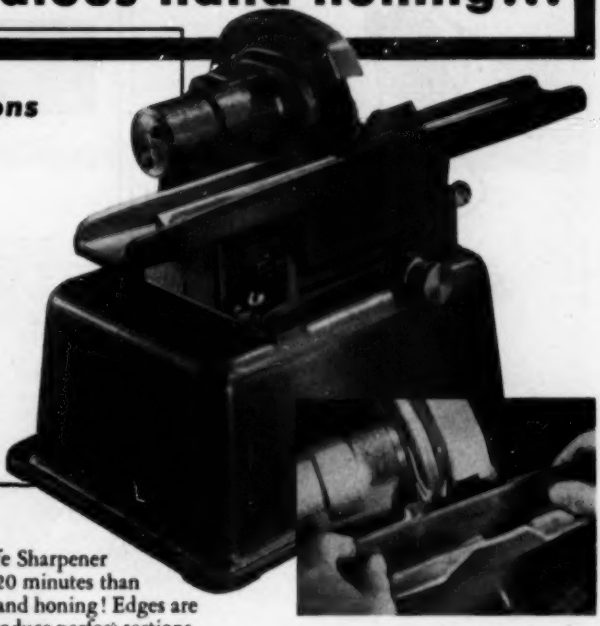

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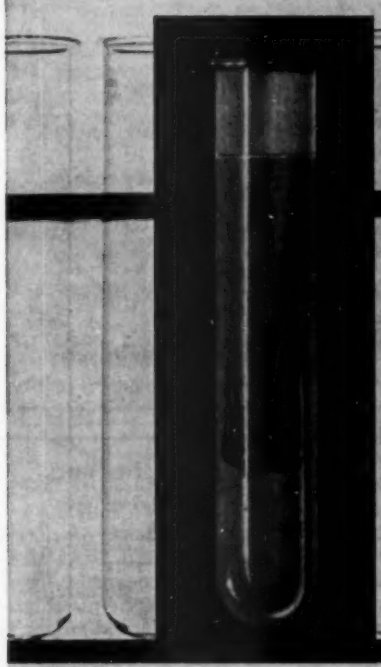

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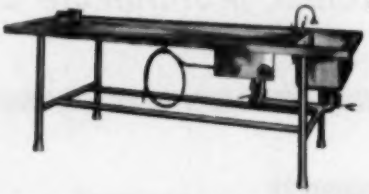

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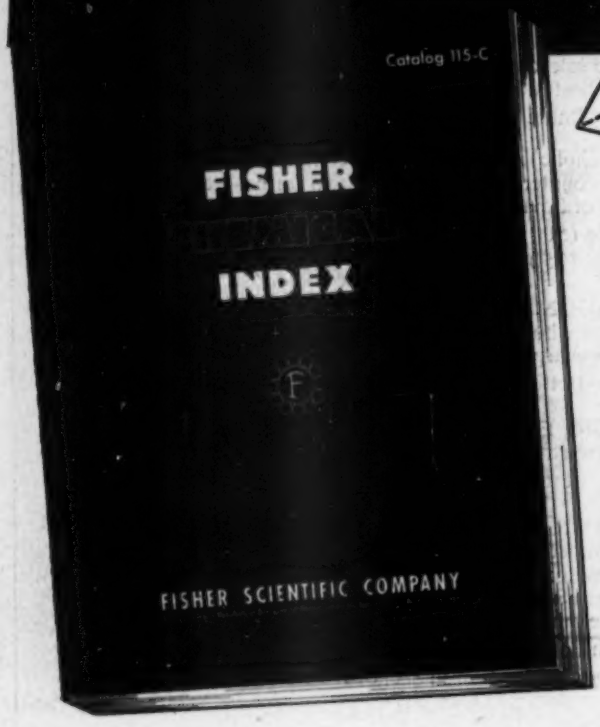

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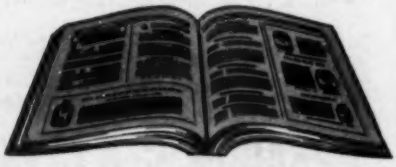

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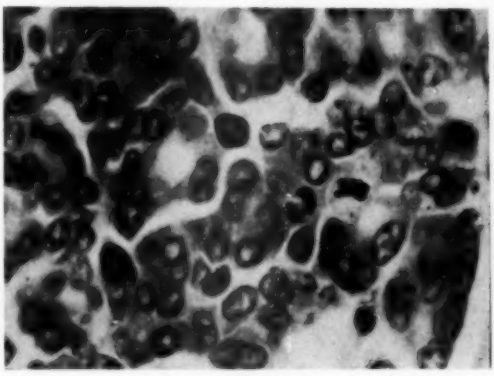

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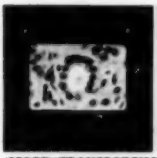

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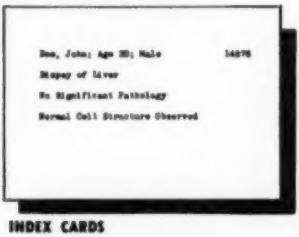

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